MONOGRAPH ON ASCORBIC ACID #116 Vol I

BIOCHEMICAL INFORMATION

SUMMARY

Acute Toxicity

The oral LD_{50}/24 hours ranges from 1000 mg/kg in the cat to 8021 mg/kg in the mouse (4496).

The subchronic toxicity ranges from LD_{50} for 7 days of 100 mg/kg in the dog to LD_{50} for 6 days of 78900 mg/kg in the guinea pig.

Short-Term Studies

Steel (8042) reported the results of a study on growth and reproduction in guinea pigs fed three levels of C; 0.4, 1.0 or 10.0 mg/100 g. He found that while at the highest level, C appeared to afford protection against reproductive malfunctions, this level was detrimental to the survival of offspring.

Concerning the question of whether there was a "hypervitaminosis C", Widenbauer (9104) concluded from his clinical observations that it did not exist as such but what was seen was a specific sensitivity shown by certain individuals to orally administered free ascorbic acid which disappeared on discontinuation of ingestion. Rietschel (6938) on the other hand considered the increase in thrombocytes following high C doses to be "curative" in sick people but "pathological" in healthy ones.

Smith (6590 and 7878) suggested the possibility that in certain "idiopathic stone-formers" very large doses of C could increase their tendency to stone formation. Poser (6590) commented that he had "used and prescribed large amounts of ascorbic acid for many years "without clinical evidence of stone formation. (Other references are in the Biochemical section.)

Long-Term Study

Korner and Weber (4496) reported the findings of an unpublished two-year feeding study in which rats received 1,

1.5 or 2 g/kg BW. All the doses were well-tolerated with no signs of toxicity and no differences were found between controls (diet only) and experimental animals. The authors noted that the highest dose corresponded to 140 g/day for a 70 kg man.

Special Studies

Schlegel et al. (7401) found that ingestion of large amounts of C prevented the carcinogenic effect of implanted 3-hydroxyanthranilic acid in mice. Alam et al. (0116) in a continuation of this research on the effect of C on bladder carcinoma found that the presence of C decreased the bladder uptake of urinary β-naphthylamine metabolites but increased their recovery from the bladder solutions. They suggested there could be implications for practical preventive measures in human carcinoma of the uroepithelium.

Greenblatt (3175) found that when twice the molar concentration of C was given to mice along with a mixture of 4-dimethylaminoantipyrine and $NaNO_2$, it prevented the formation of sufficient dimethylnitrosamine to produce hepatic necrosis. Equimolar concentrations of C gave incomplete protection.

Bourne (1032) found that guinea pigs require two mg C per day, s.c. for adequate bone regeneration and that less than 1 mg per day retarded it.

Davis and Oester (1842) found that daily concomitant administration of C s.c., inhibited arteriosclerosis induced in rabbits by epinephrine and thyroxine. The severity of the lesions was also considerably lessened. C inhibition was statistically significant and increased with increased dosage level.

Tanaka et al. (8310) showed that C was beneficial in preventing agglutination following extracorporeal circulation in dogs.

Milner (5587) concluded from the results of a controlled blind study in which adult male psychiatric patients were given either 1 g C daily or a placebo, that there was a statistically significant improvement in certain symptoms as well as in overall personality functioning.

Greenwood (3180) reported favorable results in treating patients having disc lesions with 750 to 1000 mg C daily. The postoperative course of most patients given such therapeutic C doses was also improved.

Anderson et al. (0190) reported the results of a large (818) double-blind trial in which half were given a placebo and half 1000 mg C daily for about four months. The C group was found to be less ill both with respect to number of days and severity than the placebo group. Wilson (9144) concluded from the results of a double-blind clinical test with male and female students given either dummy, 200 or 500 mg C daily that girls received more beneficial effects than boys and that for 90% of females 2000 mg C daily was required for adequate prophylaxis against the common cold as opposed to 2500 mg for males.

Briggs and Briggs (1118) concluded from examination of the effects of C supplementation on leucocyte and plasma ascorbate levels in healthy women geriatric patients and women treated with various steroid hormones, that C supplements increased leucocyte and plasma ascorbate in all groups. The largest increase was seen in geriatric and estrogen-treated persons.

Loh et al. (5007) found that leucocyte uptake of C in the presence of specific antigen was significantly less in blood from patients with atopic allergy than in control blood with added antigen. A pilot-level study (9145) showed an improvement of the immunological mechanisms when leucocytes were C saturated.

Biochemical Information

Vitamin C is absorbed efficiently; for example, about 50% of either D- or L-ascorbic acid was absorbed from the human mouth after retention for five minutes at the physiological pH of 6.0 (5001). The highest concentration of C is found in the adrenals; for example, when growing male guinea pigs were given daily intakes for 42 days, ranging from 2 to 100 mg/kg body weight, the most C was always found in adrenals, followed by spleen, brain, leucocytes, and heart (4234). In humans, females tended to retain more C than males under desaturating conditions, such as infections, and males tended to transfer more C via plasma to affected tissues (9146). During a further study of sex differences the authors concluded that females, both guinea pig and human, used C more economically than males, and also possibly synthesized small amounts under extreme duress (9150).

Metabolism and Excretion

The pathways of C biosynthesis from glucose or galactose were described by Burns (1253); the metabolic end-products of L-ascorbic acid were L-xylose, L-xylonic acid, L-lyxonic acid, and oxalic acid, and some amounts of the first three were considered to be reconverted to D-glucose via a triose pathway. The enzyme reactions studied have included L-gulonolactone oxidase, ascorbate oxidase, uronolactonase, D-glucuronolactone reductase, L-gulonate dehydrogenase, and 3-oxo-L-gulonate dehydrogenase (7316). Healthy adult men were found to excrete about 38 mg/day of oxalic acid without dietary supplements of C, and this amount did not increase significantly until C supplements reached 4 g/day or more (4705). In another study, about 40% of urinary oxalic acid came from C, other usual metabolites in urine were dehydroascorbic and 2,3-diketogulonic acids, and ascorbic acid itself was excreted when the body

store exceeded 1.5 g or plasma level exceeded 0.2 mg/100 ml; in all, about 1-3% of C intakes were excreted when intakes were minimal, and 60-80% when intakes were maximal (9146). The same author (9146)warned that unjustifiably large intakes of C could encourage the formation of oxalate stones (other such warnings will be found in Biological Data).

Effects on Enzymes and Other Biochemical Parameters

Many reports have been tabulated in the monograph; a recent summary (Wilson 9146) listed major areas of involvement as ovarian function, hemopoiesis, brain metabolism, corticosteroid release, and liver metabolism. Types of involvement included reducing action, cholesterol synthesis, fatty-acid metabolism, protein metabolism, and (via insulin) carbohydrate metabolism. For example, formation of epithelial membranes was C-dependent, and C was judged to participate in some immunological processes. The author also drew some detailed comparisons between signs of scurvy and those of the common cold. In another recent study (Gipp 2905), in piglets, C fed as 0.5% of the diet restored the absorption and utilization of iron when this was impaired by excessive copper in the diet.

Consumer Exposure Information

Controversy has existed for many years on whether avoidance of scurvy or tissue saturation is the proper criterion for daily intakes of C. In 1949 Bourne (1037) concluded that 1-2 g/day was the physiological optimum. In 1971 Hodges et al. (3624) took the opposite view, that under 10 mg/day would prevent or cure scurvy. In 1974 Wilson (9146) defined sufficiency as "necessary for normal metabolic function" and stated that this varied among individuals and from time to time, because of desaturation produced by many pathological conditions. The Recommended Dietary Allowances, from the National Academy of Sciences, National Research Council (5944), are 70 mg/day for adults and 30-80 mg/day for infants

and growing children, stated to be above the minimum for scurvy prevention and below the requirements for tissue saturation. The United States Dispensatory (6184) lists different and somewhat higher requirements, including 500 mg/day for therapeutic purposes.

Actual intakes have been estimated in the monograph as falling within these limits, but in fact are difficult to judge, partly owing to the liability of C to break down rapidly during ordinary storage, processing and preparation of foods (see monograph section on Breakdown).

CHEMICAL INFORMATION

I. Nomenclature

A. Common name: Vitamin C

B. Chemical names: Ascorbic acid; L-Ascorbic acid; L-threo-hex-2-enonic acid γ-lactone; 3-oxo-L-gulofuranolactone (enol form); L-3-ketothreohexuronic acid lactone; L-xyloascorbic acid.

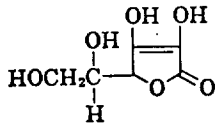
C. Trade names: Cantaxin; Cebione; Cevalin. (See original article for a more detailed list (8041).

D. Chemical Abstracts Services Unique Registry Number: 50817.

II. Empirical Formula

$C_6H_8O_6$

III. Structural Formula



IV. Molecular Weight

176.13

V. Specifications

A. Chemical

The U.S. Pharmacopeia XVIII (8613) presents the following specifications for ascorbic acid:

> Ascorbic Acid contains not less than 99.0 percent and not more than 100.5 percent of $C_6H_8O_6$.
>
> **Description:** White or slightly yellow crystals or powder. On exposure to light it gradually darkens. In the dry state, is reasonably stable in air, but in solution rapidly oxidizes. Melts at about 190°.
> **Solubility:** Freely soluble in water; sparingly soluble in alcohol; insoluble in chloroform, in ether, and in benzene.

Identification—

A: A solution (1 in 50) reduces alkaline cupric tartrate T.S. slowly at room temperature but more readily upon heating.

B: To 2 ml. of a solution (1 in 50) add 4 drops of methylene blue T.S., and warm to 40°: the deep blue color is practically completely discharged within 3 minutes.

C: Dissolve 15 mg. in 15 ml. of a solution of trichloroacetic acid (1 in 20), add about 200 mg. of activated charcoal, shake the mixture vigorously for 1 minute, and filter through a small fluted filter, returning the filtrate, if necessary, until clear. To 5 ml. of the filtrate add 1 drop of pyrrole, and agitate gently until dissolved, then heat in a bath at 50°: a blue color develops.

Specific rotation, page 936: not less than +20.5° and not more than +21.5°, determined in a solution containing 1 g. in each 10 ml.

Residue on ignition, page 901: not more than 0.1 percent.

Heavy metals, page 897—Dissolve 1 g. in 20 ml. of water, add 0.5 ml. of 0.1 *N* hydrochloric acid, and dilute with water to 25 ml.: the heavy metals limit is 20 parts per million.

Assay—Dissolve about 400 mg. of Ascorbic Acid, accurately weighed, in a mixture of 100 ml. of water and 25 ml. of diluted sulfuric acid. Titrate the solution at once with 0.1 *N* iodine, adding 3 ml. of starch T.S. as the end-point is approached. Each ml. of 0.1 *N* iodine is equivalent to 8.806 mg. of $C_6H_8O_6$.

Packaging and storage—Preserve in tight, light-resistant containers.

B. Food Grade

The Food Chemicals Codex, Second Edition (1645), gives the following specifications for food grade ascorbic acid:

Assay. Not less than 99.0 percent of $C_6H_8O_6$.

Specific rotation, $[\alpha]_D^{25°}$. Between +20.5° and +21.5°.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Residue on ignition. Not more than 0.1 percent.

TESTS

Assay. Dissolve about 400 mg., accurately weighed, in a mixture of 100 ml. of water, recently boiled and cooled, and 25 ml. of diluted sulfuric acid T.S. Titrate the solution immediately with 0.1 *N* iodine, adding starch T.S. near the end-point. Each ml. of 0.1 *N* iodine is equivalent to 8.806 mg. of $C_6H_8O_6$.

Specific rotation, page 939. Determine in a solution containing 1 gram in 10 ml. of water.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. A solution of 1 gram in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Loss on drying, page 931. Dry at 105° for 3 hours.

Residue on ignition, page 945. Ignite 1 gram as directed in the general method.

Packaging and storage. Store in well-closed, light-resistant containers.

Functional use in foods. Nutrient; dietary supplement.

VI. Description

A. General Characteristics

Ascorbic acid is a white or slightly yellow crystalline solid or powder which gradually darkens on exposure to light.

B. Physical Properties

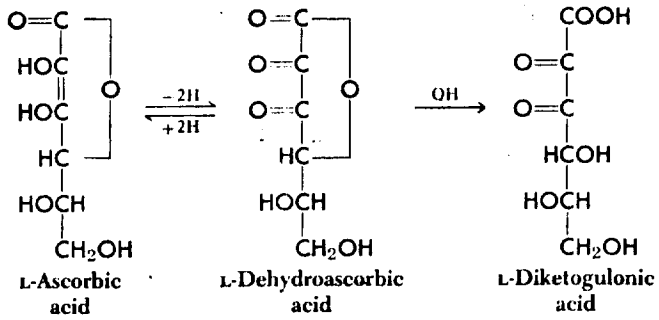
The compound is quite soluble in water (1 g/3 ml), somewhat soluble in alcohol (1 g/30 ml), but insoluble in chloroform, ether, and benzene. It is insoluble in fats and oils.

Physical Constants:

Density:	1.65
Melting point:	190-192°C (some dec.)
Specific rotation:	+20.5° to +21.5° (c = 1)

C. Stability in Containers, etc.

Ascorbic acid is readily oxidized to dehydroascorbic acid, especially in the presence of small concentrations of metal ions. Iron and copper are particularly effective catalysts. The reaction is reversible. (9097)



L-Ascorbic acid — L-Dehydroascorbic acid — L-Diketogulonic acid

In alkaline solutions, ascorbic acid is very rapidly oxidized by air and the reaction is not metal catalyzed (2690). When dry, it is reasonably stable to air but oxidizes and gradually darkens on exposure to light in impure preparations (1645). It has been stated that crystalline ascorbic acid can be stored under normal laboratory conditions for years with little change in activity (2690). Ascorbic acid is heat-stable in the absence of oxygen and other oxidizing agents (2690).

Dangerously high pressures may develop in ampuls containing solutions of 10% or more of ascorbic acid during normal storage due to decomposition in which carbon dioxide is produced (6184).

Ascorbic acid should be stored in tight, light-resistant containers (8613).

VII. Analytical

The 11th edition of AOAC (0339) gives two methods for C determination:

(1) 2,6-Dichloroindophenol method. This method is not applicable in the presence of Fe^{2+}, Sr^{2+}, Cu^{+}, So_2 sulfite or thiosulfate. This method is suitable for fruit and vegetable juices.

(2) Microfluorometric method. Suggested for C in pharmaceutical products and gelatin-encapsulated pharmaceuticals.

Table 1 lists analytical methods suitable for C determination in a wide variety of foods as well as pharmaceuticals.

VIII. Occurrence and Levels

A. Plants

Vitamin C occurs naturally in many plants, especially in the fruits. The West Indian cherry (Malpighia punicifolia L.) is reported to be one of the world's richest sources of C with a content of 1,000 to 1,400 mg per 100 g (0329). Other rich sources of C are peppers, the citrus fruits, tomatoes, cabbage, parsley, watercress, fresh spinach, rutabaga, leaf lettuce, strawberries, and acerola juice. Potatoes are an important dietary source in many countries (2690). The levels for orange, grapefruit, and lemon juice, etc., are given in Table 2 (3271). The values for a large variety of other fruits and vegetables are presented in Tables 3 and 4 (0753,7018).

Table 1. Analytical Methods for Ascorbic Acid

Year	Substance(s) Analyzed	Method	Reagent(s)	Biblio
1939	Raw and commercially pasteurized milk	colorimetric	indophenol/acetate	9176
1940	Evaporated milk, powdered milk & products	colorimetric	H_2S/indophenol	9175
1941	Citrus juices	titrimetric	iodate	0172
1941	Citrus fruit juices in presence of SO_2	direct titrimetry	dichlorophenol/ indophenol	4367
1941	Milk, fruit juices, urine	titrimetric	B.Coli/indophenol	3271
1942	Beans & cabbages	colorimetric	metaphosphoric acid/ indophenol	5711
1942	Citrus fruit	potentiometric titration	indophenol/iodate	6764
1944	Dehydrated & raw cooked fruits & vegetables	"continuous-flow" titrimetry	indophenol	3409
1944	Various plant tissues	photometric	thiourea/2,4-dinitrophenylhydrazine	7018
1945	Highly colored food	photometric	xylene/peroxide/ formaldehyde/indophenol	6989
1945	Fruits & vegetables	polarographic	K biphthalate/phosphate buffer	2859
1945	Food extracts	electrometric	indophenol	4927
1945	Tomato & tomato juice	extraction/ titrimetry	xylene/indophenol	5966
1947	Canned foods (Fe contaminant)	titrimetric	indophenol	3738
1947	Fresh & cooked vegetables; caramelized dried vegetables	"continuous-flow" titrimetry	indophenol	3410

Table 1 . Analytical Methods for Ascorbic Acid (Cont'd)

Year	Substance(s) Analyzed	Method	Reagent(s)	Biblio
1956	Orange juice, honey, urine	colorimetric	$FeCl_3$/αα'-dipyridyl	8185
1957	Flour	spot test	ferric-ferricyanide	3483
1958	Fruit products	titrimetric with polarized Pt electrodes	indophenol	1741
1960	Vitamin mixture	paper chromatography	ammoniacal silver nitrate	2703
1962	Frozen peas	colorimetric	2,6-dichlorobenzenone indophenol	8128
1964	Highly colored fruit or veg. juices. Drug mixtures.	potentiometric titrimetry	N-bromosuccinimide	3386
1964	Colored fruit juice syrups	titrimetric	$HgCl/I_2$ (soln)/sodium thiosulfate/starch	4627
1966	Canned fruit juices (in presence of Fe (II)& Sn (II) salts)	titrimetric	EDTA/indophenol	6364
1969	Vitamin capsules & tablets	gas chromatography	N,O-Bis (trimethylsilyl) acetamide	7567
1970	Evaporated milk	chromatographic separation of 2,4-dinitrophenyl-hydrazine derivatives	2,4-dinitrophenyl-hydrazine	8496
1970	Pharmaceutical preparations	colorimetric	2,3 5-triphenyltetrazolium chloride	3446
1971	Ascorbic acid	titrimetric	ferroin sulfate/cerium (14) sulfate	9462
1971	Bread improver formulations	titrimetric	2,4-dinitrophenyl-hydrazine	0580

Table 1. Analytical Methods for Ascorbic Acid (Cont'd)

Year	Substance(s) Analyzed	Method	Reagent(s)	Biblio
1971	Ascorbic acid (in presence of reducing sugars)	colorimetric	chloroauric acid	3445
1972	Citrus fruits	spectrophotometric/ reduction	Iron (III)	3951
1972	Orange juice	photometric	methylene blue	9101
1972	Ascorbic acid in fruits & vegetables	oxidimetric titration (no interference by tenfold excess of oxalic citric, tartaric, succinic, malic and acetic acids and glucose, fructose and sucrose)	K hexacyanoferrate (III) in acid medium	7321

The contents of true vitamin C and "apparent vitamin C" of a number of fruits, vegetables, and food products are given in Table 5 (9190). The term "apparent vitamin C" refers to substances that may be found in natural and processed foods which resemble vitamin C very closely in chemical and physical characteristics but do not have antiscorbutic properties. They are not differentiated from true vitamin C by the indophenol titration as usually applied and consequently may cause the results to be too high (9190).

Table 2. Reduced and Total Vitamin C in Commercial Canned Juices (3271)

The values are given in mg. per cc. of undiluted juice.

Juice	pH, undiluted juice	0.2 N NaOH to bring 1 cc. to pH 6.2	Immediately on opening can		After aeration and 5 days storage in glass at 1°			
			Ascorbic acid	Total vitamin C	Ascorbic acid	Total vitamin C	Total vitamin C after addition of 0.335 mg. dehydroascorbic acid	Recovery of added dehydroascorbic acid
		cc.						per cent
Orange	3.8	0.48	0.393	0.394	0.364	0.395	0.716	95.8
Grapefruit	3.8	0.92	0.344	0.342	0.307	0.321	0.652	98.9
Lemon	2.5	4.35	0.330	0.330	0.170	0.302	0.635	99.4
Pineapple	3.6	0.53	0.084	0.092	0.075	0.084	0.419	100
Apple	3.5	0.46	0.004	0.006	0	0.016	0.349	99.4
Sauerkraut	3.9	0.36	0.153	0.007	0.037	0	0	0
Composite vegetable	4.4	0.22			0.013	0	0.031	9.3

Table 3 . The Vitamin C Content of Various Foods per Hundred Grams* (0753)

Food	Mg. of Ascorbic Acid	International Units
Apples		
Delicious	2.5	50
Jonathans	2.5	50
McIntosh	2	40
Winesaps	5	100
Golden delicious	4	80
Yellow Newton	5	100
Dried	0	0
Juice	See type of apple	
Apricots, fresh	1	20
Apricots, dried	8	160
Asparagus	20	400
Bananas	8	160
Beans		
Green	10	200
Green, canned	4	80
Sprouted	10	200
Dried	0	0
Beef, muscle, cooked	Trace	Trace
Beer	0	0
Beets	5	100
Beet leaves	35	700
Blackberries, fresh	3	60
Blackberries, frozen	3	60
Blueberries, low bush	4	80
Blueberries, high bush	10	200
Broccoli	50	1,000
Brussels sprouts	50	1,000
Butter	0	0
Cabbage, young, green	40	800
Cabbage, old	20	400
Cantaloup	7	140
Carrots	3	60
Cauliflower	30	600
Celery stalk	5	100
Chicory	10	200
Chard, Swiss, stalks	5	100
Chard, Swiss, leaves	20	400
Cheese, all varieties	0	0
Cherries, sweet	8	160
Cider, fresh	See apples	
Chicken meat, cooked	Trace	
Cod liver oil	0	0
Collard	60	1,200
Corn		
Sweet	10	200
Canned	6	120
Dried	0	0
Cranberries	10	200
Cucumbers	2	40
Currants, black	100	2,000
Currants, red	15	300
Dandelion greens	40	800
Dates, cured	0	0
Egg plant	5	100
Eggs	0	0
Endive	10	200
Escarole	7	140
Figs, fresh	2	40
Figs, dried	0	0
Fish, fresh cooked	Trace	Trace
Fruit juice	See fruit	
Gooseberries	25	500
Grain, dried, all varieties	0	0
Grass, fresh, green	60	1,200
Grape juice	Trace	Trace
Grapefruit juice, fresh	40	800
Grapefruit juice, canned	30	600
Horseradish	100	2,000
Jelly	0	0
Kale	50	1,000
Kohlrabi	70	1,400
Leek	15	300
Lemon juice		
Fresh	60	1,200
Cold storage	40	800
Canned	50	1,000
Lettuce, green leaf	10	200
Lettuce, head	5	100
Lime juice, fresh	30	600
Liver, beef, cooked	10	200
Malt	0	0
Milk		
Human	6	120
Cow's, raw, fresh	2	40
Cow's, pasteurized	0 to 1	0 to 20
Cow's, dried	5	100
Mustard leaves	60	1,200
Nuts, all kinds	0	0
Okra	10	200
Onion	10	200
Orange juice, fresh	50	1,000
Orange juice, canned	45	900
Orange syrup	0	0
Papaya	40	800
Parsley	175	3,500
Parsnips	5	100
Peas, green	15	300
Peas, canned	5	100
Peaches, fresh	7	140
Peaches, dried	25	500
Pears, fresh	3	60
Pear juice, canned	Trace	Trace
Peppers, green	180	3,600
Peppers, red, ripe	230	4,600
Pineapple, fresh	25	500
Pineapple, canned	10	200
Plums	2	40
Potatoes, new	15	300
Potatoes, old	7	140
Prunes	2	40
Prunes, dried	0	0
Pumpkins	5	100
Quince	5	100
Radishes	12	240
Raisins	0	0
Raspberries	15	300
Rhubarb	15	300
Rutabaga	20	400
Seeds, mature, dry (all varieties)	0	0
Spinach, fresh	60	1,200
Spinach, canned	5 to 10	100 to 200
Sauerkraut juice	0 to 5	0 to 100
Squash	5	100
Strawberries	25	500
Sweet potatoes	8	160
Swede	See rutabaga	
Tangerines	30	600
Tomatoes		
Green	20	400
Vine ripe	30	600
Artificial ripe	25	500
Tomato juice, fresh	30	600
Tomato juice, canned	25	500
Turnips	30	600
Turnip greens	60	1,200
Vegetable juices canned	0 to 5	0 to 100
Watercress	50	1,000
Watermelon	5	100
Yeast	0	0

* Unless otherwise indicated, these values are on fresh foods.

Table 4. Comparative Analyses of Plant Tissues for Vitamin C (7018)

The values are expressed as mg. per 100 gm. or 100 cc. of material.

Tissue	Ascorbic acid	Ascorbic acid + dehydroascorbic acid	Dehydroascorbic acid	
	Indophenol photometric method (1)	2,4-Dinitrophenylhydrazine method (2)	By difference (2) − (1) (3)	Direct 2,4-dinitrophenylhydrazine (4)
Apple	3.1	4.5	1.4	0.0
Rhubarb	1.6	5.0	3.4	0.4
Lemon juice	57.6	59.5	1.9	0.5
Potato	28.1	31.6	3.5	0.5
Turnip	27.2	30.0	2.8	0.7
Asparagus	19.7	20.8	1.1	1.0
Carrot	5.4	8.3	2.9	1.0
Peach	1.3	3.3	2.0	1.0
Plum	1.2	3.6	2.4	1.2
Pineapple	15.6	21.0	5.4	1.3
Green peas	17.5	19.6	2.1	1.3
Cucumber	2.2	5.5	3.3	1.4
Grapefruit juice	40.3	45.0	4.7	1.6
Spinach	54.0	54.0	0.0	1.8
String beans	16.0	16.4	0.4	1.9
Lima beans	39.6	43.5	3.9	2.0
Lime juice	31.0	37.5	6.5	2.0
Simlin	14.7	17.4	2.7	2.5
Orange juice	69.0	74.0	5.0	3.4
Potato, dehydrated	99.4	111.0	11.6	5.0
Green pepper	169.0	173.0	4.0	5.2
Cauliflower	75.5	72.5	−3.0	6.3
Yellow squash	16.0	26.0	10.0	7.5
Parsley	209.0	219.0	10.0	8.3
Orange peeling	266.0	282.0	16.0	11.3
Grapefruit peeling	196.0	235.0	39.0	14.0
Kale	234.0	238.7	4.7	17.5
Broccoli	108.0	115.0	7.0	18.0
Cabbage, dehydrated	391.8	405.0	13.2	18.7
" "	512.0	540.0	28.0	22.5
Lemon peeling	204.0	210.0	6.0	25.0
Orange marmalade	21.2	23.0	1.8	2.4
" "	4.4	7.1	2.7	0.8
Apple butter	0.8	2.6	1.8	1.8

Table 5 . Apparent and True Vitamin C Contents of Foods, etc. (9190)

Material	pH of material	Vitamin C content (mg./100 g.) Total	Apparent (a)	True	True as percentage of total
Fresh fruit and fruit preparations:					
Black currants, fresh	3·2	202	4	198	99
Black currant juice	3·2	140	5	135	96
Black currant syrup, freshly made	—	103	2	101	98
Black currant syrup after 8 weeks at room temp. in dark	—	96	4	92	96
Black currant syrup after 13 weeks at 27° in dark	—	58	3	55	94
Black currant syrup after 10 weeks at 37° in dark	—	68	8	60	88
Rose hip syrup after 12 months at 0–5°	3·7	156	20	136	88
Rose hip syrup after 12 months	3·6	101	69	32	31
Cherry juice, conc., after 5 years at 0–5°	3·8	47	45	2	4
Lemon juice, conc., after 18 months at 0–5°	1·7	193	42	151	78
Lemon juice, conc., after 4 years at 0–5°	1·7	27	21	6	22
Walnuts, unripe (*Juglans regia*), 2–3 g.	—	2619	1310	1309	50
Walnuts, unripe, 6–7 g.	5·2	2961	987	1974	67
Walnuts, unripe, 15–20 g.	3·9	2028	408	1620	80
Walnuts, unripe, after precipitation of tannin (*b*)	3·9	1909	400	1509	79
Dried fruits and vegetables:					
Dried bananas (*c*)	5·0	6	1	5	83
Dried carrots (*c*)	4·8	90	49	41	45
Dried potatoes (*d*)	4·5	27	4	23	85
Dried rose hip extract (mean of five samples) (*e*)	4·1	1300	50	1250	96
Dried spinach (*c*)	7·0	2	0·5	1·5	75
Dried tomatoes (*c*)	4·7	89	55	34	38
Malt extracts:					
Liquid malt extract, low diastatic power (D.P.)	4·7	3	(*f*)	0	0
Liquid malt extract, normal D.P.	4·7	12	(*f*)	0	0
Liquid malt extract, normal D.P.	4·8	5	(*f*)	0	0
Liquid malt extract after ½ hr. at 100°	4·8	12	(*f*)	0	0
Liquid malt extract after 2 hr. at 100°	4·8	20	(*f*)	0	0
Liquid malt extract after 9 months at 37°	3·6	103	(*f*)	0	0
Dried malt extract, low D.P. (*g*)	4·7	3	(*f*)	0	0
Dried malt extract, normal D.P. (*g*)	4·7	12	(*f*)	0	0
Sugars and molasses:					
Sugar, white (mean of three samples)	—	0	0	0	0
Sugar, brown (mean of four samples)	—	1	1	0	0
Golden syrup	—	27	27	0	0
Molasses, beet	—	18	18	0	0
Molasses, cane	—	11	11	0	0
Molasses, before charcoal treatment (*h*)	5·6	46	46	0	0
Molasses, after charcoal treatment	6·2	19	19	0	0
Molasses, beet concentrated preparation	5·4	222	(*d*)	0	0
Molasses, beet concentrated preparation	—	304	(*d*)	0	0
Molasses, cane concentrated preparation	—	196	(*d*)	0	0
Cocoa, chocolate:					
Cocoa, defatted, mixed sample	7·0	65	65	0	0
Cocoa, defatted, after precipitation of tannin	7·0	12	(*d*)	0	0
Chocolate, vitaminized	6·1	44	8	36	82
Chocolate, vitaminized	5·7	36	4	32	89
Miscellaneous:					
Beer, light draught	4·6	0·3	0·3	0	0
Beer, dark draught	4·4	1	1	0	0
Parsley (*i*)	7·0	220	40	180	82

Notes

(*a*) Apparent vitamin C determined by method of Wokes *et al.* [1943*b*] checked occasionally by Mapson's method [1943*b*].
(*b*) By method of Mirimanoff & Mori [1940].
(*c*) After about 4 years' storage in air-filled containers at room temperature.
(*d*) After 6 weeks' storage in air-filled containers at room temperature.
(*e*) pH range 4·0–4·2.
(*f*) Some of the dye reductant in these preparations reacted with HCHO under the given conditions, but since it had been produced by heating was considered not to be ascorbic acid.
(*g*) Prepared by evaporation *in vacuo* of the corresponding liquid malt extract.
(*h*) Treatment normally used in refining sugar.
(*i*) During extraction cyanide used to inhibit action of oxidizing enzymes and stabilize apparent and true vitamin C.

B. Animals

The usual body store of Vitamin C in a human adult is about 4 g according to Lowry et al. (5041). The cortex of the adrenal glands and the corpus luteum contain the highest concentration of any tissue in the body (1.4 to 2.3 mg/gram) according to the analyses of Bessey and King (0178,0914). The brain, liver, testes, ovaries, and other glandular tissues contain considerably less with values of 0.1-0.4 mg/g (0914). The minimum normal blood plasma level is 0.8 mg per 100 ml (6184). Milk from a well-nourished mother contains about 5.2 mg/100 ml (6184). Cow's milk is relatively low; Kon and Watson report a reduced C content of about 2 mg/100 ml (0178, 4479). Meats and eggs contain very little (0178). (See Biochemical Aspects, for more detailed information.)

C. Synthetics

Ascorbic Acid Tablets, Ascorbic Acid Injection, and a variety of other preparations are used in human and veterinary nutrition and medicine (see original articles for details). (6184, 8041, 8613)

D. Natural Inorganic Sources

None.

I. Acute Toxicity

A. Six Species of Mammals

Korner and Weber (4496) in their review of C tolerance reported the results of acute toxicity studies carried out by a number of researchers. These are summarized in Table 6 and Table 7 .

Table 6 . Acute Toxicity (LD_{50}/24 hours) (4496)

Animal species	Mode of administration (mg/kg)				Literature reference
	Oral	Subcutaneous	Intravenous	intraperitoneal	
Mouse	8021	5000	1058	2000	Bachtold, H.P., Demole, V., 1934, Gaudiano, et al.
Rat	>5000	5000	1000		Demole, V., 1934
Guinea pig	>5000	>1000	500	2000	Demole, V., 1934
Rabbit	>2000	>1000	>1000	>1000	Demole, V., 1934
Cat	>1000	> 500	> 500		Demole, V., 1934
Dog	>5000	> 200	> 200		Demole, V., 1934

Table 7 . Subchronic Toxicity (LD_{50}) (4496)

Animal species	Mode of administration (mg/kg/day)				Duration (days)	Literature reference
	Oral	Subcutaneous	Intravenous	intraperitoneal		
Mouse	8021				10	Bachtold, H.P.
Mouse			1058		10	Bachtold, H.P.
Rat	> 6500				6	Lang, K.
Rat		> 600			28	Gineste, P.J.
Guinea pig	> 8900				6	Lang, K.
Guinea pig		500			7	Demole, V. 1935
Guinea pig				100	16	Villard, P. et al
Rabbit			500		7	Demole, V. 1935
Rabbit				100	16	Villard, P. et al
Cat			> 500		9	Edwards, W.C.
Dog	100				7	Demole, V. 1935
Dog			> 2000		3	Leveque, J.I.

B. Human

In 1972, Severova (7581) reported the case of a 42-year-old woman who was sensitized to a number of drugs and died of anaphylactic shock (on the seventeenth treatment day) after the seventh i.m. injection of C (1 ml of a 5 percent solution per injection).

II. Short Term Studies

A. Rats

1. Korner and Weber (4496) refer in their review of C tolerance to the work of Kieckenbush et al. (1964, ref.no.81 in original paper) and Lang (1965, ref. no. 88 in original paper) who carried out separate six-week long toxicity feeding studies with rats. No injurious effects were reported for daily p.o. administration of 6.5 g/kg BW (based on initial weight of animal), with respect to weight gain, mortality, protein efficiency, food intake and histopathology; no differences were noted between experimental and control animals.

Daily doses greater than 25 g/kg BW were found to be toxic. The authors concluded from their data that the maximum non-toxic dose was 10 g/kg BW. They related this to man by noting on this basis that a daily dose of 1 g C (equivalent to 14 to 15 mg/kg BW) afforded a safety factor of 1:700.

2. Because "large doses" of C had been previously shown to terminate pregnancy and cause stillbirth in guinea pigs, rats, and mice (Samborskaya, references cited in original paper,(7253),in 1966, Samborskaya and Ferdman (7253) investigated C-related hormonal disturbances in pregnant rats.

Three groups of sexually mature female albino rats (180 g) on an ordinary diet were injected s.c. with:

Group 1 (13), 25 i.u. folliculin in vegetable oil per rat.

Group 2 (14), 150 mg neutralized C per rat.

Group 3 (17 controls), physiological saline.

The results showed:

(1) Group 1: Eleven terminated pregnancies (day 8-11).

(2) Group 2: Three terminated pregnancies (day 13-15).

(3) Group 3: No terminated pregnancies.

(4) Dead fetuses or indications of abortion were found in the uteruses of animals with terminated pregnancies.

(5) Cytological smears (Papanicolau stain) from:

(a) Groups 1 and 2 both contained numerous squamous epithelial and nucleated cells.

(b) Group 2 contained fewer leucocytes than Group 1.

(c) Group 3 contained very few isolated nucleated and leucocyte cells and no epithelial cells.

The authors concluded that both the termination of pregnancy and the cytological similarity with folliculin-treated animals could be explained by an increased estrogen level following administration of large C doses.

3. In 1966, Lippi et al. (4967) examined the hepatic cells of guinea pigs treated with massive doses of C.

Ten male Wistar rats (200-300 g) were injected i.m. with 2.5 mg C in physiological saline daily for 5 days. Five controls received physiological saline only. The animals were sacrificed and the livers fixed (see original paper for details).

The lysosomes in livers of the treated animals appeared under the electron microscope to be increased in number and volume with lesions in the cellular membranes. A significant increase in protein synthesis with changes in the amount and distribution of ribosomal RNA was also observed.

4. In 1970, de Albuquerque and Henriques (1863) investigated the growth of rats (no specifications given) on diets enriched with C. The results are summarized in Table 8 .

It was observed that:

(a) Doses of one percent had no effect on growth.

(b) With five percent doses, there was an initial weight loss later restored.

(c) With ten percent doses, there was physical debility sometimes leading to death within a few days. There were no macroscopically visible signs of visceral injury. The only notable change was liquid colon contents accompanied by gaseous distention.

The authors postulated that the reduction in growth might be attributable to a laxative effect and impaired absorption of food.

Table 8 . Effects of Vitamin C Doses on Weight Change in Rats (1863)

Weight Changes of Rats Fed a Normal Diet

Weight, g \ Rat	1	2	3	4	5	6
Initial	72	70	80	65	70	65
After 3 days	90	85	87	88	100	83
After 6 days	100	100	104	100	117	100

Weight Changes of Rats Fed a Diet Containing 1% C

Weight, g \ Rat	1	2	3	4	5	6
Initial	80	80	80	80	80	80
After 3 days	80	85	78	80	80	78
After 6 days	85	85	87	85	83	87
After 9 days	90	90	90	90	95	95

Weight Changes of Rats Fed a Diet Containing 5% C

Weight, g \ Rat	1	2	3	4	5	6
Initial	85	75	85	75	85	55
After 3 days	80	75	82	70	80	55
After 6 days	75	75	90	70	80	55
After 9 days	85	80	100	68	84	61

Table 8. Effects of Vitamin C Doses on Weight Change in Rats (1863) (Cont'd)

Weight Changes of Rats Fed a Diet Containing 10% C

Weight, g \ Rat	1	2	3	4	5	6
Initial	60	65	68	65	65	80
After 3 days	52	64	68	52	64	80
After 6 days	Died	69	67	Died	57	69

B. Guinea Pigs

1. Korner and Weber (4496) refer in their review of C tolerance, to the work of Diacono in 1935 (ref. no. 35 in original paper) who gave young guinea pigs daily parenteral doses of 100 mg/animal (200 to 500 mg/kg BW) over a four-month period. Growth development and weight gain were normal. The skeletal system appeared normal under X-ray examination. The symptoms noted were; a slight discoloration of the sclera; alteration in hair growth; diarrhea;and a relative lowering of the blood Ca level.

More recent studies (1964 and 1965) reported (Kieckebusch et al., ref. no. 80 and Lang ref. no. 88 in original paper) that daily doses of 8.9 g/kg BW for 14 weeks were non-toxic to guinea pigs.

2. In 1968, Steel (8042) reported the results of a study on growth and reproduction of C fed guinea pigs. Three levels of C; 0.4, 1.0, or 10.0 mg/100 g BW were fed to 14-day old female guinea pigs until they had produced and weaned three litters. The female offspring were fed the same level of C as the parents.

The observations were:

(1) First generation offspring were heavier at both 14 and 92 days old, gaining more weight in the 78-day growth period than the parent animals.

(2) At the lowest C level (0.4 mg/100 g BW), starting pregnancy and maintaining pregnancy were more difficult, and abortion rate and death rate were higher than in the other C supplemented animals (1.0 and 10.0 g/100 g BW).

(3) When parents were fed 0.4 mg/g BW, the survival rate of offspring was highest. It was lowest when parents were fed 10 mg/100 g BW.

The author concluded from the data that:

(1) The highest level of C appeared to protect the parent animal from obvious malfunctions of the reproductive faculty.

(2) A high C intake by the parent animal is detrimental to the survival of offspring despite a lack of obvious signs in the parent animal of physiological effects.

C. Rabbits

Korner and Weber (4496) refer in their review of C tolerance, to the work of Diacono in 1935 (ref. no. 35 in original paper) who gave young rabbits daily parenteral doses of 100 mg/animal (200 to 500 mg/kg BW) over a four-month period. Growth and weight gain were normal and no organ pathology was observed. Transient subjunctival skin hemorrhages were the only symptom found.

D. Humans

1. In 1936, Widenbauer (9104) considered the question of whether there was clinical evidence for hypervitaminosis C. He concluded from his observations that:

(a) Only particularly sensitive adults showed such toxic effects as nausea, retching, a sensation of heat, hot flushes in the head, a reddening of the face, headache, fatigue, exhaustion, disturbed sleep, diarrhea and increased peristalsis, increased diuresis, reduction of the pulse-rate by 6 to 10 beats per minute. In sensitive infants the toxic symptoms noted were: increased dermography, skin hyperemia, rubella-like morbilliform or scarlatiniform erythemas and increased peristalsis.

(b) Toxic symptoms generally disappeared after discontinuation of C with no permanent injury.

(c) There were no toxic effects when sensitive individuals were administered the Na salt p.o. or i.p. rather than free ascorbic acid.

(d) Hypervitaminosis C as such does not exist rather what is seen is a specific sensitivity shown by certain individuals to orally administered free ascorbic acid which disappears on discontinuation of medication (cf. this section below, D2).

2. In 1939, Rietschel also reviewed the question of whether there was a "hypervitaminosis C" and made the following points: (6938)

(a) High p.o. doses of C (ca. 200 mg daily) to infants produced an increase in blood platelets (see Table 9).

(b) In some cases 200 mg doses daily apparently caused restlessness, sleeplessness or diarrhea.

(c) Papayanopulos and Schroeder (see 6268,section B IV, I,3) had found an increase in blood platelets in ill adults following "high vitamin C enrichment". The author accedes that whereas in these cases an increase in thrombocytes could be considered a "curative effect", in the case of healthy infants it is pathological.

(d) The author's observations with adults including himself administered 500 mg of C daily for 5 days were increases in both thrombocytes and insomnia, which disappeared on withdrawal.

(e) The author concluded that "large unphysiological doses of synthetic ascorbic acid can provoke symptoms of pathological reaction in adults and children." (cf. this section above, D1).

Table 9. Effects of Vitamin C on Thrombocyte Count in Infants (6938)

No.	Patient	Daily vitamin C dose, mg	Thrombocytes/ml (counted according to Fonio)	Time of Determination
1	Otto V., 6 Mon.		124000	before the test
		100	118000	5-6 days
		200	194000	10-14 = for 5 days
		100	168000	15-17 = for 3 days
		200	202000	18-23 = for 6 days
			132000	after 3 days
2	Irmgard R., 3 1/2 mon.		126000	before the test
		100	127000	3-9 = for 7 days
		200	218000	10-14 = for 5 days
		100	166000-	
			180000	15-17 = for 3 days
		200	224000	18-23 = for 6 days
			140000	after 3 days
3	Gerlinde D., 3 Mon.		265000	before the test
		100	220000-	
			268000	8-15 = for 8 days
		200	326000-	
			340000	16-18 = for 3 days
			196000	after 2 days
4	Ingrid F., 3 Mon.		183000	before the test
		100	191000	for 8 days
		200	472000	for 3 days
			191000	
5	Harald Schrb. 2 Mon.		114000	before the test
		100	124000	for 8 days
		200	288000	for 3 days
			112000	
6	Adelheid G.,		140000	before the test
7	Helga Sch., 4 Mon.		204000	before the test
		200	312000	after 12 days
8	Hans Kn., 4 Mon.		193000	before the test
		200	320000	after 12 days

3. In 1952, Lowry et al. (5040) investigated the effect in humans of prolonged high dosage with C. One woman and three men were each administered a 1000 mg daily supplement of C in three divided doses (with meals) for three months.

Measurements of the following were made at appropriate intervals:

(1) The C concentration in the serum,

(2) The concentration in the white blood cells plus platelets,

(3) The C tolerance curve,

(4) The urinary output of C.

The data for the four subjects agreed so closely that they were averaged together (see Table 10).

It can be seen from Table 10 that there was no progressive change in any of the observed parameters. No harmful effects were observed during the three-month intake period.

The authors concluded that prolonged high dosage of C had no qualitative or quantitative effect on the manner of disposal of excess acid by the body.

Table 10. The Ascorbic Acid of Urine, White Cells, and Serum of 4 Subjects Receiving 1000 mg Ascorbic Acid per Day (5040)

Day of exp	Urine mg/day	White cells* mg %	Serum after 400 mg test dose (mg %) 0	1 hr	3 hr	5 hr	7 hr
0		27±2†	1.22±.09	1.78±.13	1.97±.11	1.80±.06	1.63±.04
5	817 #±35†	28±3	1.81±.09	2.45±.09	2.39±.08	2.14±.11	1.94±.04
21	804 ±25	30±1	1.79±.06	2.28±.11	2.35±.05	2.19±.11	2.03±.04
39	714 ∫±35	28±1	1.82±.13				
55		28±2	1.54±.08				
67		31±1	1.93±.15	2.45±.17	2.48±.20	2.19±.24	2.09±.26
98	822 ±47	28±1	1.64±.10	2.24±.99	2.14±.08	1.83±.13	1.76±.06

*White blood cells+platelets. †±Stand. error. #3rd day of exp. ∫43rd day of exp.

4. In 1964, Fazio (2436) reported the case of a male 4-year-old child who had swallowed 20 pills containing about 10 g of C. Observed for 18 hours, he showed no signs of toxicity, either gastric or acidic and remained in good spirits and health.

5. Hoffer (3628) in a letter to the New England J. Med. in 1971 reported on the use of "megadoses of C,3 to 30 g per day since 1953" with about 1000 largely schizophrenic patients. He stated that he has not observed a single case of: kidney stone formation, miscarriage, excessive dehydration or any other "serious toxicity". Diarrhea has been the most notable side effect observed and is ameliorated by reducing the dosage by one or two g.

The author stated an "impression" that such megadoses of C reduced cold frequency.

6. In 1972, Smith et al. (7878) suggested that very large doses of C may lead to acquired hyperoxaluria by degradation of the C to oxalate. (For comments see letter by Poser and response by Smith, this section D,7 following.)

7. Poser (6590) in a letter to the New Eng. J. Med. in 1972 commented that he had "used and prescribed large amounts of ascorbic acid for many years" without any clinical evidence of oxalate stone formation. He was responding to the suggestion of a relationship between ingestion of large amounts of C and nephrolithiasis which had been made in a paper (this section, D,6 above). One of the paper's authors, Smith, responded by noting that in certain "idiopathic stone-formers," the fact that L-ascorbic acid is a precursor of urinary oxalate which adds to the already elevated oxalate levels in their urine could increase their tendency to stone formation. (See also Biochemical Information III 5-8)

8. In 1973, Vickery (8788) reported the case of a 66-year-old woman with one kidney (the other removed due to an occluding calculus) and a family history of kidney problems, who developed an intestinal obstruction at the ileocecal valve following massive intake (4500 mg daily) of C for four or more days when ill and regular intake of 300 mg daily. Surgery revealed the presence in the ileum of numerous hard objects which on analysis were found to consist largely of ascorbic acid.

Table 19. The Comparative Effects of Ascorbic Acid, Aspirin, and Phenylbutazone in Animal Models of Inflammation (2100)

Test	Percent of Inhibition		
	Ascorbic Acid	Aspirin	Phenylbutazone
UV Erythema	0	80±1.5	90±1.3
Carrageenan	12±1.1	29±2.5	49± .6
Adjuvant Arthritis			
7 days	21± .1	12± .2	33± .1
14 days	25± .2	0	43± .1

F. Guinea Pigs

1. In 1942, Bourne (1032) attempted to ascertain the critical value for the amount of C required to promote optimum regeneration of bone.

Experimental Procedure:

Young male guinea pigs (28) were first placed on a scorbutic diet then divided and given various s.c. doses of C daily as follows:

5 received 0.25 mg
4 received 0.50 mg
5 received 1.00 mg
5 received 2.00 mg
4 received 4.00 mg

After one week, a hole (mm in diameter was bored in both femora of each guinea pig. The animals were killed after 7 days. Histological examination showed:

III. Long-Term Study

In 1972, Korner and Weber (4496) reviewed the literature concerning tolerance for high dosages of C. They reported the unpublished results of a two-year feeding study by Surber and Gerioli (ref. no. 151 in original paper) in which three groups of 26 rats per group (m and f) were fed with their diet 1, 1.5 or 2 g/kg BW C for two years. Controls received the diet only.

All the doses were equally well tolerated with no indications of toxicity. When compared to controls, no differences were noted with respect to growth development mortality rates, hematology, blood chemistry, histological examinations.

The authors concluded that the highest tolerated dose in the rats, 2 g/kg/day would correspond in a 70 kg man to 140 g/day.

IV. Special Studies

A. In Vitro

1. In 1948, Shapiro (7623) investigated the influence of C on cell division. Eggs of the sea urchin, Arbacia punctulata, were exposed to concentrations of C in sea water ranging from 2.62×10^{-6} M to 1.57×10^{-3} M. The rate of cell division was measured as the time for 50 percent of the cells to undergo first cleavage.

The results are shown in Figure 1. It can be seen that C delays cell division at all concentrations above a minimal one. At very high C concentrations cell division can be inhibited in 100 percent of the cells. No concentration at which cleavage was accelerated was found.

2. In 1967, Walker et al. (8966) attempted to show whether the exposure of cells to C would increase their resistance to infection with viruses. The strains of viruses used are shown in Table 11. Immediately before use, C was added to the tissue culture medium at a concentration of 10 mg C/100 ml medium.

The experimental results are shown in Table 12. There was no apparent antiviral effect. (For experimental details see original paper.)

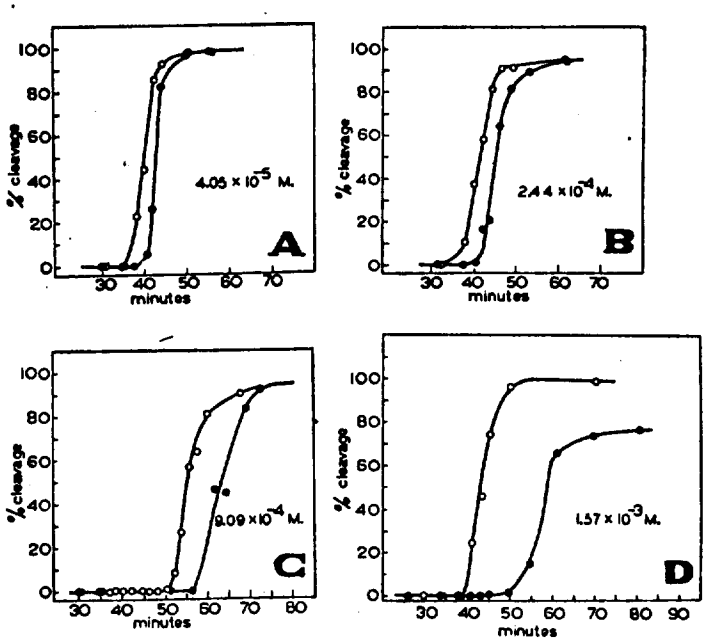


Figure 1. A, B, C, and D show the increasing retardation of cell division with increasing concentration of vitamin C. The concentrations are given to the right of each pair of curves. Open circles, controls; closed circles, eggs in sea water with vitamin C. (7623)

Table 11. Viruses Used in Tissue-culture Experiments with Ascorbic Acid (8966)

Family	Group	Strain	Tissue Used for Propagation
Picornavirus ..	Rhinovirus 11	16/60	Human embryo lung
" ..	" 11	HBB	" " "
" ..	" M	HGP	" " "
Myxovirus ..	Influenza B	B Eng/101/62	Monkey kidney
" ..	Parainfluenza 3	Prototype	" "
" ..	Respiratory syncytial	Randall	HeLa
Adenovirus ..	Adenovirus 5	Prototype	"
Enterovirus ..	Poliovirus 1	LSc 2ab	Human embryo lung
" ..	E.C.H.O. 11	U virus	" " "
" ..	Coxsackie A21	Local strain	" " "
Herpesvirus ..	Herpes simplex	Perrett*	" " "

* Albanese *et al.* (1966).

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VOLUME 1

GRAS MONOGRAPH SERIES

ASCORBIC ACID

prepared for
THE FOOD AND DRUG ADMINISTRATION
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B. Tadpoles

In 1970, Galea et al. (2719) studied the antitoxic effect of C against poisoning with the organomercury compound, phenylmercuriborate (PMB). Frog tadpoles, Rana temporaria - 3 to 6 weeks old were divided into experimental groups of 25 to 30, in tap water solutions of PMB (30 ml/tadpole) of 6 different concentrations from 2.5×10^{-6} M/liter to 7.8×10^{-9} M/liter with and without simultaneous addition of C (100 mg %). The C was added both concomitantly with the PMB solution (as phenosept) and after the appearance of poisoning.

The authors concluded from their observations that C increased the resistance of tadpoles to the toxic effect of PMB.

C. Newts

In 1970, Wirl and Seilern-Aspang (9164) found that in two strains of newts studied, the occurrence of skin carcinoma (both spontaneous and induced by wounding) was seasonal reaching a maximum from January to March and a minimum by April and May. This was inversely related to the C content of the kidneys (where it is synthesized by the urodeles), the mass of granulation tissue in the wounds and the collagen content of the skin.

The authors speculated from their observations that the decrease in collagen synthesis in the dermis associated with C deficiency leads to an uncontrolled (malignant) epidermal growth after wounding.

D. Mice

1. In 1960, French and Freedlander (2620) showed in a preliminary experiment that C can mitigate carcinogenesis in mice. Two weeks after 43 mice (female, strain A, 4 mos.) received 0.5 percent C (pH5) in their drinking water, they were administered 1 mg/g mouse urethan i.p. along with 82 controls. All the animals were sacrificed after 11 weeks and pulmonary adenomas counted visually. The average number of tumors per mouse was: controls, 7.77 ± 0.39; C treated, 5.17 ± 0.48. The observed difference and P values for controls vs. C treated were, 2.60, $P<0.00005$. All mice remained healthy and there were no observable abnormalities on autopsy.

Table 12. Minimal Infectious Dose of Virus for Tissue Cultures Maintained in Medium Containing Ascorbic Acid (8966)

Virus	Infectious Dilution (TCD_{50}) in Titrations Performed in Cultures Treated with

Infections	Low C might lower natural resistance, and infections might increase the demand for C; reports were cited for colds and other respiratory infections and rheumatic fever, but not polio.
Wound healing	The authors reviewed reports since 1928 and concluded that a role of C in collagen synthesis was relevant to this and healing of bone fractures.

3. In 1939, Papayanopulos and Schroeder (6268) studied the effect of "large doses" of C (as Merck's Cebion) on thrombocytes in patients with various diseases. Patients were administered C i.v. in amounts ranging from a total of 3000 to 6000 mg over a period of several days.

The results which are summarized in Table 25 show that there was a regular tendency for the thrombocyte count to be increased above normal values. Patients receiving smaller C doses i.v. (100 to 300 mg daily for several days) showed no clearcut trend in their thrombocyte count. When a single 500 mg injection was given, a temporary increase in the thrombocyte count was seen after 15 minutes which reverted to normal after 75 minutes (see Figure 5).

Table 25. Effects of C on Thrombocytes in Patients with Various Diseases (6268)

No.	Diagnosis	Vitamin C-dose	Thrombocytes in ccm, according to Fonio	Thrombocytes in ccm, according to Flossner	Time of Determination
1	Cachexia	3000 mg (daily 500 mg)	65000 150000 196000		a b h
2	Dry pleurisy	3500 mg (daily 500 mg)	260000 500000 455000		a c h
3	Liver cirrhosis	3000 mg (daily 500 mg)	88000 277000 396000		a b g

H. Dogs

1. In 1963, Tanaka et al. (8310) evaluated the effect of C as an anti-sludging agent in the microcirculation of the dog. A radio-isotope method which quantifies erythrocyte agglutination was used.

The results showed that agglutination was strikingly diminished in the C-treated animals ($p<0.001$) and was not statistically different from the agglutination due to anesthesia alone.

The authors concluded that C was beneficial in preventing agglutination following extracorporeal circulation in dogs.

I. Humans

1. In 1936, Hagen (3313) reported that the clinical and hematological condition in severe benzene poisoning markedly improved when 75 to 100 mg of C were administered daily.

2. In 1938, Abt and Farmer (0046) reviewed the list of conditions reported as responding to C therapy or prophylaxis:

Scurvy & "latent scurvy"	C was specific and protective.
Anemia	C was reported to enhance Fe absorption.
Hemorrhage	The authors concluded that C was ineffective unless the hemorrhage was related to scurvy.
Teeth & gums	Unanimity that C was needed for maintenance, but dearth of therapeutic evidence in detail.
Eye	An association of C deficiency with cataract.
Skin	Doses of 300-450 mg were reported to depigment skin, observed by the authors in Addisonian patients including one Black person; C might be specific in lupus erythematosus.
Thyroid	Data on interactions with C were contradictory.
Gastric ulcer	Patients were reported as liable to C deficiency.
Immunity	C, i.v., appeared capable of stimulating antibody production, and of protecting against anaphylaxis, but evidence was incomplete.

Table 23. Effect of Ascorbic Acid on the Recovery of Radioactivity from the Urinary Metabolites of ^{3}H-β-Naphthylamine by the Bladder Solution (0116)

	With ascorbic acid (W AA)			Without ascorbic acid (W/O AA)			
Replicate	Radioactivity administered (DPM)	Average % recovered	Standard deviation (% recovery)	Radioactivity administered (DPM)	Average % recovered	Standard deviation (% recovery)	Comparison of means (W AA)—(W/O AA) (sign only)
1	22.21×10^6	76.6 (2)[a]	24.25	30.78×10^6	29.3 (2)[b]	11.88	+
2	28.07×10^6	73.2 (3)	1.37	28.32×10^6	67.7 (3)	9.44	+
3	28.61×10^6	33.9 (3)	9.54	25.09×10^6	30.2 (2)[b]	14.43	+
4	32.14×10^6	69.4 (2)[b]	13.22	30.84×10^6	50.5 (3)	4.24	+
5	35.32×10^6	58.2 (3)	2.85	35.06×10^6	53.1 (3)	0.95	+
6	40.99×10^6	68.4 (3)	8.61	40.99×10^6	63.2 (3)	5.00	+
7	23.51×10^6	39.8 (3)	3.71	18.81×10^6	32.5 (3)	5.28	+
8	22.42×10^6	35.0 (3)	7.40	20.70×10^6	27.9 (3)	7.37	+
Overall average	29.16×10^6	56.8 (22)		28.82×10^6	44.4 (22)		$p = 0.004$ for the Sign Test

[a] Number in parentheses is the number of rabbits used in each treatment.

[b] Indicates an observation missing due to the death of a rabbit before completion of the experiment from causes unrelated to the treatment administered.

Table 24. Effect of Ascorbic Acid on the Uptake of Radioactivity from the Urinary Metabolites of ^{3}H-β-Naphthylamine by the Bladder Tissues (0116)

	With ascorbic acid (W AA)			Without ascorbic acid (W/O AA)			
	Radioactivity recovered[a]		Standard deviation	Radioactivity recovered		Standard deviation	Comparison of average %
Replicate[c]	Av DPM/g	% Adm dose[b]	(% recovery)	Av DPM/g	% Adm dose	(% recovery)	(W AA)—(W/O AA)
3	133×10^3 (3)	0.466	0.173	129×10^3 (2)[d]	0.512	0.135	—
4	183×10^3 (2)[d]	0.568	0.107	539×10^3 (3)	1.74	1.15	—
5	273×10^3 (2)[d]	0.772	0.19	313×10^3 (3)	0.892	0.03	—
6	155×10^3 (3)	0.378	0.15	181×10^3 (3)	0.442	0.172	—
7	26×10^3 (2)[d]	0.11	0.03	51×10^3 (3)	0.271	0.11	—
8	30×10^3 (3)	0.13	0.085	55×10^3 (3)	0.267	0.086	—
Overall average	133×10^3 (15)	0.388		211×10^3 (17)	0.698		$p = 0.02$ For the Sign Test

[a] Administered doses are the same as those in Table I.

[b] DPM/g/Total adm. dose × 100.

[c] In replicates 1 and 2, no determination was made for radioactivity uptake.

[d] Indicates missing observation.

3. In 1973, Alam et al. (0116) continued their research into the effect of C on bladder carcinogenicity (see this section D, 3 and 4) investigating the effect of C on the bladder reabsorption of β-naphthylamine metabolites. When ingested orally, β-naphthylamine is a proven potent carcinogen in man, dogs, and nonhuman primates (references are given in the original paper).

The experiment was carried out by first administering p.o. a single dose of tritium labeled β-naphthylamine to female dogs (5 kg) and extracting the urinary metabolites. The reabsorption of the metabolites by rabbit bladders (male rabbits, 2 to 3 kg) was then investigated.

The effect of C on the recovery of radioactivity in the bladder solutions and uptake by the rabbit bladder tissues after in vivo impregnation of urinary metabolites of ^{3}H-β-naphthylamine is summarized in Table 23. Table 24 shows that in the presence of C, there was less radioactivity taken up by the tissues. From the data it can be seen that:

(1) The presence of C decreased the bladder uptake of the urinary β-naphthylamine metabolites,

(2) but increased their recovery from the bladder solutions.

The authors suggest that:

(1) C could have a preventive effect on bladder carcinoma resulting from β-naphthylamine administration because the decreased uptake of the carcinogenic compound by the bladder would reduce its potency.

(2) These findings are further substantiation of their hypothesis that the urinary environment, especially its redox potential, may be a significant factor in the modification of the carcinogenicity of a compound.

(3) There may be important implications for practical preventive measures in human carcinoma of the uroepithelium.

Table 22. Results of Experiment on Administration of C to Rabbits (8856)

Rabbit	1	2	3	4	Average values
			1st test (control)		
Erythrocytes (E)	5,840,000	5,720,000	6,200,000	5,800,000	5,890,000
Leukocytes (L)	c,100	8,000	9,200	8,900	8,500
ESR	3.5	3	3	2	2.9
Dry Residue (DR)	10	12.5	11.9	11.7	11.5
Electrophoretic albumin ratio (EAR)	50.4	51.2	48.9	52.7	50.8
Globulins - alpha	7.7	7.1	6.4	6.1	
beta	17.1	12.9	10.9	9.9	48.1
gamma	25.2	30.1	29.5	27.8	
			2nd test (100 mg/kg vitamin C)		
Erythrocytes (E)	6,250,000	6,000,000	6,240,000	5,970,000	6,115,000
Leukocytes (L)	9,700	8,500	9,000	9,400	9,150
ESR	4	5	4.5	6	4.9
Dry Residue (DR)	11	12	12	13.2	12
Electrophoretic albumin ration (EAR)	42.7	37.2	39.1	29.9	37.2
Globulins - alpha	10.5	7.9	9.2	12.5	
beta	20.1	18.5	17.5	10.7	62.3
gamma	30.1	40.1	38.7	38.3	
			3		
			3rd test (12 hours later)		
Erythrocytes (E)	6,200,000	6,100,000	6,200,000	6,120,000	6,155,000
Leukocytes (L)	10,000	8,900	9,900	10,700	9,870
ESR	5	5	6	6	5.5
Dry Residue (DR)	10.2	11.9	12.1	12.3	11.6
Electrophoretic albumin ration (EAR)	28.5	33.4	30.5	30.1	30.8
Globulins - alpha	10.9	9.8	10.1	9.5	
beta	20.4	19.5	18.5	13.7	68.8
gamma	40.3	39.9	43.1	38.3	
			4th test (24 hours later)		
Erythrocytes (E)	5,900,000	6,190,000	6,290,000	6,200,000	6,145,000
Leukocytes (L)	10,000	9,100	9,700	10,900	9,920
ESR	3	3	3.5	2.5	3
Dry Residue (DR)	10.5	12	11.7	11.2	11.3
Electrophoretic albumin ration (EAR)	57.2	51.3	59.2	49.5	45.3
Globulins - alpha	7.2	7.1	9.8	8.6	
beta	17.1	19.3	13.7	10.0	45.5
gamma	22.5	20.5	23.8	25.6	

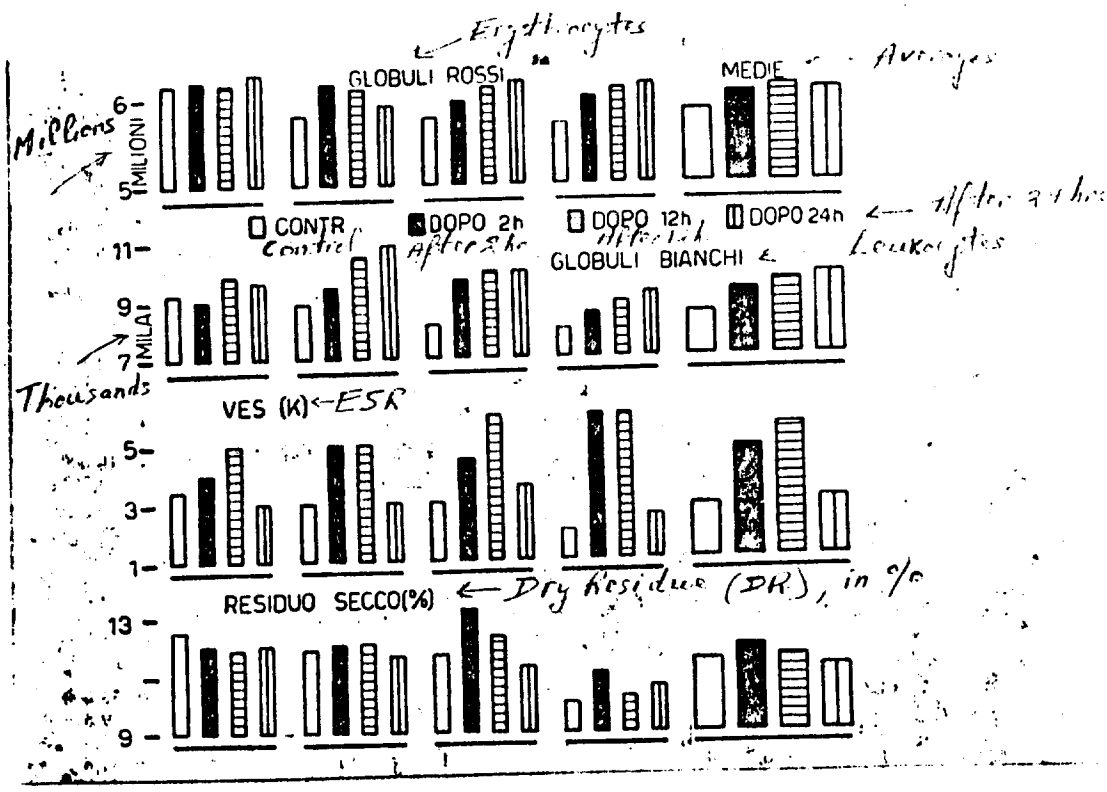

Figure 4. Results of Experiment on Administration of C to Rabbits (8856)

Table 21. Significance of Influence of Ascorbic Acid on Epinephrine-Thyroxine Sclerosis (1842)

Treatment*	No.with sclerosis/ total no.animals	% incidence	Probability	Severity factor(% incidence x avg deg.of sclerosis)
E + T (C)**	18/20	90		90 x 2.45 = 220
C + A, 50 mg/kg	6/9	66	>.2	66 x 1.66 = 109.5
C + A, 100 mg/kg	11/18	61	<.01	61 x 1.5 = 91.5
C + A, 500 mg/kg	5/9	55	>.1	55 x 1.22 = 67.1

* Probability compared to controls, a value of <.05 is assumed significant. Probability: (1) for total inositol group = >.1: (2) for total ascorbic acid group = <.01. Animals whose death occurred before 6th day of treatment were not included in biometrical analysis.

** E = Epinephrine; T = Thyroxine; C = Controls; A = Ascorbic acid

2. In 1957, Volterrani and Baiotti (8856) studied the possible changes in blood composition when C was administered to rabbits. Four male rabbits from the same breeding and on the same diet had their blood withdrawn at four intervals; (1) during fasting; (2) two hours after i.m. administration of 100 mg/kg C; (3) 12 hours later; (4) 24 hours later.

The results are summarized in Figure 4 and Table 22.

It was observed that:

(1) There were no appreciable changes in the erythrocytes and leucocytes.

(2) A definite increase in the ESR and total globulins, particularly the gamma fraction was seen at 2 and 12 hours following C administration which returned to normal at 24 hours.

(3) The blood sedimentation rate was higher at 2 and 12 hours following C administration.

C was administered s.c. to 36 animals starting 3 days before epinephrine and thyroxine and continuing for 15 days for a total of 18 days as follows: 18 received 100 mg/day; 9 received 500 mg/day; and 9 received 50 mg/day. A summary of the experimental protocol is given in Table 20 and an analysis of the data in this table is given in Table 21.

The results showed that daily concomitant administration of:

(a) 50 mg C lowered the sclerosis to 66 percent

(b) 100 mg C lowered the sclerosis to 61 percent

(c) 500 mg C lowered the sclerosis to 55 percent.

The severity of the lesions produced was also considerably lessened. The C inhibition was statistically significant.

Table 20. Influence of Ascorbic Acid on Epinephrine Thyroxine Sclerosis (1842)

Treatment*	No.of Rabbits	Deaths before 6 days	Deaths 6 to 15 days	No.animals sacrificed on 16th day	Degree of sclerosis 0	1	2	3	4
E + T (C)**	25	5	12	8	2	4	4	3	7
C + A, 50 mg/kg	12	3	5	4	3	2	1	1	2
C + A, 100 mg/kg	22	4	11	7	7	2	4	3	2
C + A, 500 mg/kg	10	1	6	3	4	1	2	2	0

* Probability compared to controls, a value of <.05 is assumed significant. Probability: (1) for total inositol group = >.1: (2) for total ascorbic acid group = <.01. Animals whose death occurred before 6th day of treatment were not included in biometrical analysis.

** E = Epinephrine; T = Thyroxine; C = Controls; A = Ascorbic acid

The observations were:

(a) The C-deficient animals showed an average of 50.3 percent more fat in their livers than the C-treated group.

(b) The fatty degeneration was relatively mild in the pigs receiving C compared with the C-deficient animals.

The author concluded that C protected against hepatic damage.

3. In 1963, Schmidt et al. (7418) investigated the C content of the transplanted Daels-sarcoma in guinea pigs with that of the organ tissue in both normal and tumor-bearing animals. The animals were administered 50 mg daily either p.o. or i.m. of a proprietary C preparation starting with three days prior to the i.m. Daels-sarcoma transplant.

It was observed that:

(a) Tumor tissues showed a higher total C content than liver ($P<5\%$) kidneys and brain.

(b) The C content in tumor animal liver was lower than in the normal liver ($P<5\%$). (See this section p.000 for similar findings in the rat.)

(c) The number of sarcomas and their size was not significantly affected by C administration rather a tendency for tumor growth promotion was noted.

4. In 1965, Guirgis (3270) reported finding that injected C (i.p. or i.m.) at the dose levels used had a protective effect against histamine shock and anaphylactic shock in the guinea pig. No protective effect was found when C was administered p.o. or 6 hours after injection (for experimental details see original paper).

G. Rabbits

1. In 1952, Davis and Oester (1842) investigated whether C would affect epinephrine-thyroxine induced arteriosclerosis in rabbits. Combined i.v. injections of epinephrine and thyroxine produced aorticsclerosis characterized by lack of atheroma and the presence of severe medical and intimal sclerosis.

Summary of Changes

Dose of Vitamin C mg	Periosteal Reaction	Endosteal Reaction	Fibres in Hole	Trabeculae
None	Negative	Negative	A few	None
0.25	Slight multiplication of cells in cambial layer	Negative	Fairly numerous	None
0.50	Considerable multiplication of cells in the cambial layer	Slight reaction. Beginnings of formation of trabeculae	Numerous	None
1.00	Cambial layer forming trabeculae	Small number of trabeculae forming	Very large numbers	A few
2.00	Very large reaction. Enormous multiplication of cambial layer cells. Many trabeculae formed	Extensive formation of trabeculae	Very large numbers	Numerous particularly in inner part of hole
4.00	Ditto	Ditto	Ditto	Ditto

Using the amount of trabeculae formed in the bored hole at the end of one week as indication of the effect of C on bone regeneration, the author concluded that:

(1) Guinea pigs require 2 mg C/day by injection for adequate bone regeneration.

(2) Less than 1 mg C/day seriously retarded bone regeneration in the guinea pig.

2. In 1943, Beyer (0767) investigated whether C would protect guinea pigs on a scorbutogenic diet against hepatic damage. Guinea pigs on a C-deficient diet (250 to 400 g) were divided into two groups: (a) 10 animals were injected with 30 mg C s.c. daily; (b) 18 animals received no C. After 18 days both groups were injected with 25 mg/kg BW hydrazine sulfate.

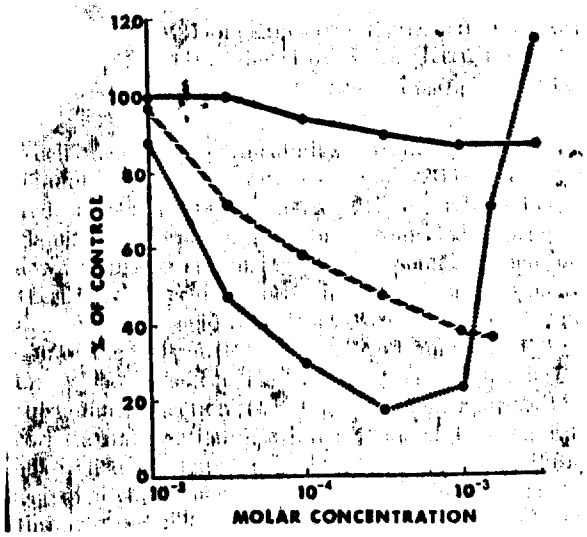

Figure 2. The Inhibition of Lysosomal β-glucuronidase by Ascorbic Acid (●·····●) Aspirin (●——●), and Phenylbutazone (●–·–·–●). The Drugs Were Added in 50 µl Aliquots to 1 ml of Dilute Liver Lysosome Suspension in KRP. (2100)

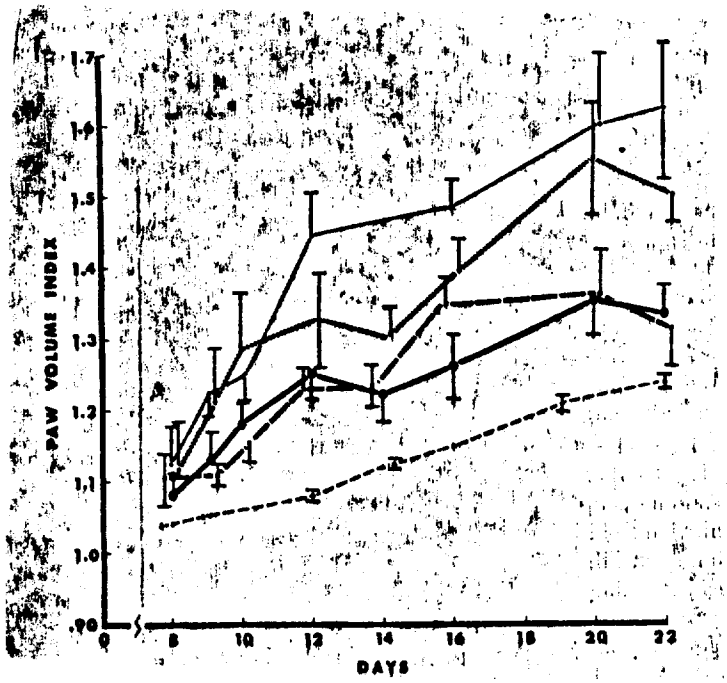

Figure 3. Inhibition of Swelling by Ascorbic Acid (●——●), Aspirin (●·····●), and Phenylbutazone (– – – –) in Peritonitis-Polyarthritis. Control, Noninjected, (Δ– – –Δ). Control, TB Injected (○——○). Paw Volume Index-Paw Volume/Initial Paw Volume. Standard Error of the Mean is Presented (2100)

It was observed that:

(1) The total C content of the tumor tissue was higher than that of the examined parenchymatose organs of the tumor rats (liver, brain, testicles, spleen), $P<5\%$.

(2) The total C content in the liver, spleen and brain of the tumor rats was lower than in the normal rats.

The authors concluded that the increased C in the tumors was "at least partially at the cost of the vitamin supply to other organs".

2. In 1972, Dolbeare and Martlage (2100) investigated the effect of C in several animal models of inflammation. Their report compared the inhibitory actions of C, aspirin and phenylbutazone against rat liver lysosomal β-glucuronidase (in vitro), UV erythema in guinea pigs carageenan paw edema in rats, adjuvant arthritis and peritonitis-polyarthritis in rats.

The results are shown in Figures 2 and 3 and Table 19. (For experimental details see original paper.)

It was found that C was:

(1) A very effective inhibitor of β-glucuronidase activity and peritonitis-polyarthritis swelling.

(2) A moderately effective inhibitor of adjuvant arthritis swelling.

(3) Weakly inhibitory in carageenan paw edema.

(4) Not inhibitory against UV erythema.

tertiary amines is blocked by C. The in vivo findings showed that twice the molar concentration of C prevented the formation of sufficient DMN to produce hepatic necrosis but equimolar concentrations of C gave incomplete protection. When C was given along with DMN, the hepatoxic effect was neither potentiated nor antagonized.

Table 18. Experimental Design and Results of Treatment* (3175)

Group	$NaNO_2$	AP	Ascorbic Acid	DMN	Hepatic Necrosis†
1	58	-	-	-	0
2	-	17.3	-	-	0
3	-	-	114	-	0
4	58	17.3	-	-	+++
5	58	17.3	114	-	0
6	58	17.3	57	-	+
7	-	-	114	1.6	+
8	-	-	-	1.6	+

*All values are in moles of compound given by gavage. Total volume was 0.4 ml/mouse; each group had 4 mice.

†Measured semiquantitatively 48 hours after gastric intubation.

+:Individual cells necrotic about central vein; ++: all cells around central vein necrotic and one-third of lobule involved; +++: pronounced centrilobular necrosis involving more than one-third of lobule.

E. Rats

1. In 1963, Schmidt et al. (7418) compared the C content of various transplanted tumors in rats with the content of the parenchymatose organs of both tumor-infected and normal animals. The experiments were carried out with 64 tumor-bearing (5 different transplanted tumors) and normal rats.

The results (see Table 17) indicated that all three of the test materials showed "a fairly strong sarcoma-180 hindering potentiality". Cupric ions enhanced inhibition by C.

Table 17. Antitumour Activity of Enediols (9262)

Agent	No. of mice	Place of injection	Dose in each injection (mg/kg)	Times of injection	Average Tumour weight (g)	Inhibition ratio (%)
Ascorbic acid (AA)	10	Subcutaneous	150(AA)	5	2.1	46.5
Ascorbic acid + $CuSO_4$	10	Subcutaneous	150(AA) + 5.4(Cu)	5	1.2	69.2
Control	10	Subcutaneous	—	5	3.9	—
Dehydro-ascorbic acid	10	Subcutaneous	120	6	0.5	88.1
Control	10	Subcutaneous	—	6	4.2	—
2,3-Diketo-gulonic acid	10	Subcutaneous	115	5	2.0	54.5
Control	10	Subcutaneous	—	5	4.4	—

6. In 1971 Schatz and Lal (7369) reported that C (12 mg/kg) given i.v. to mice protected them against brain damage from hyperbaric oxygen mediated via GABA levels. Pargyline and succinic acid also gave protection but probably not via the same pathway.

7. In 1973, Greenblatt (3175) investigated the effect of varying concentrations of C on preventing hepatic necrosis in mice when administered along with mixtures of 4-dimethylaminoantipyrine (AP) and sodium nitrite ($NaNO_2$). The solutions, 17.3 μ moles of AP and 58 μ moles of $NaNO_2$ were administered by gavage to groups of four male NZO/B1 mice (20 to 30 g) as shown in Table 18. These doses produce acute centrilobular necrosis of the liver in 48 hours due to dimethylnitrosamine formation. Varying concentrations of C were given along with the carcinogenic mixture. The amounts and results are shown in Table 18.

The author concluded that his data confirmed the in vitro findings of Mirvish et al. (Science 177:65-67, 1972) that the nitrosation of several secondary and

4. In 1970, Schlegel et al. (7401) continued the investigations in their laboratory (see above abstract,) to determine whether C protected against bladder tumor formation from bladder implantation of the carcinogenic orthoaminophenol, 3-hydroxyanthranilic acid (3-HOA), a tryptophan metabolite. The experimental protocol is summarized in Table 16. A total of 310, 60 to 120 day-old Swiss albino female mice were used. Compound 3 which was tested also is an oxidative product of 3-HOA.

Table 16. Data summary on the effects of ascorbic acid, compound 3 and 3-hydroxyanthranilic acid and their interactions on the course and development of bladder tumors in mice. (7401)

Group	Chemicals Implanted In Cholesterol Pellets Into Mice Bladders	Ascorbate Fed In Drinking Water	No. Mice Treated Originally	No. Mice Which Survived 40 Weeks	Tumors Observed In 40 Weeks		
					Pre-Malignant	Malignant	Total
1	Cholesterol alone	None	58	49	3	2	5
2	Cholesterol alone	250 mg.%	33	23	1	2	3
3	Compound 3*	None	56	39	0	2	2
4	Compound 3*	250 mg.%	42	28	1	2	3
5	3-HOA†	None	70	46	1	8	9
6	3-HOA†	250 mg.%	51	37	1	2	3

* 3-hydroxy-5-carboxy-benzoquinone-(2-hydroxy 6-carboxy-anil)-(1) imide (4).
† 3-hydroxyanthranilic acid.

The results showed that group 5 (3-HOA implants) had a significantly higher incidence of bladder tumor formation than any other group and that administering C (group 6) prevented the 3-HOA from exerting any specific carcinogenic effect.

The authors concluded that the high urinary ascorbate levels due to p.o. ingestion of large amounts of C, an anti-oxidant, prevented the carcinogenic effect of 3-HOA by preventing its oxidation to a carcinogenic compound.

5. In 1971, Yamafugi et al. (9262) investigated whether C inhibited tumor growth. The implanting material was ascites taken from a male ddN-mouse 7 to 9 days after injection with sarcoma - 180. A solution of C was injected s.c. on alternate days from 30 hours after implant for two weeks. Controls received Ringer solution. Similar experiments were also carried out with dehydroascorbic acid and 2,3-diketogulonic acid, metabolic products of C.

Table 14. Results in Mice Treated with Ascorbic Acid and Challenged with Influenza A Virus (8966)

Treatment		Dilution of Virus	
		10^{-3}	10^{-4}
Ascorbic acid	Lung lesion score* ..	12/16	3/16
	Deaths	2/4	0/4
Saline	Lung lesion score ..	6/16	3/16
	Deaths	1/4	0/4

* Slightly modified from the method of Horsfall (1939).

3. In 1969, Pipkin et al. (6496) investigated the effect of elevated urinary levels of C in mouse urine on the carcinogenicity of 3-hydroxyanthranilic acid (3-HOA) implanted into mouse bladders. A 2 x 2 factorial experiment was designed to test the interaction of C and 3-HOA on: (a) survival of mice, (b) the number of induced malignant tumors and (c) the total number of induced tumors.

A summary of the experiment which used 60 to 120 day old Swiss albino female mice is shown in Table 15. All surviving mice were killed at 40 weeks following surgical implantation. Analysis of the data showed that C inhibited the anticipated carcinogenicity of 3-HOA.

Table 15. Data Summary of the Effects of Ascorbic Acid and 3-Hydroxyanthranilic Acid and Their Interactions on the Development of Bladder Tumors in Mice (6496)

Group	Chem. implanted in cholesterol pellets into mouse bladders	Ascorbate fed in drinking water (mg/100 ml)	No. of mice treated orig.	No. that survived 40 wks	Tumors observed in 40 wks: Pre-malig.	Malig.	Total
A	Cholesterol alone	None	58	49	3	2	5
B	Cholesterol alone	250	33	23	1	2	3
C	3-HOA[a]	None	70	46	1	8	9
D	3-HOA	250	51	37	1	2	3

[a] 3-Hydroxyanthranilic acid

B. Tadpoles

In 1970, Galea et al. (2719) studied the antitoxic effect of C against poisoning with the organomercury compound, phenylmercuriborate (PMB). Frog tadpoles, Rana temporaria - 3 to 6 weeks old were divided into experimental groups of 25 to 30, in tap water solutions of PMB (30 ml/tadpole) of 6 different concentrations from 2.5×10^{-6} M/liter to 7.8×10^{-9} M/liter with and without simultaneous addition of C (100 mg %). The C was added both concomitantly with the PMB solution (as phenosept) and after the appearance of poisoning.

The authors concluded from their observations that C increased the resistance of tadpoles to the toxic effect of PMB.

C. Newts

In 1970, Wirl and Seilern-Aspang (9164) found that in two strains of newts studied, the occurrence of skin carcinoma (both spontaneous and induced by wounding) was seasonal reaching a maximum from January to March and a minimum by April and May. This was inversely related to the C content of the kidneys (where it is synthesized by the urodeles), the mass of granulation tissue in the wounds and the collagen content of the skin.

The authors speculated from their observations that the decrease in collagen synthesis in the dermis associated with C deficiency leads to an uncontrolled (malignant) epidermal growth after wounding.

D. Mice

1. In 1960, French and Freedlander (2620) showed in a preliminary experiment that C can mitigate carcinogenesis in mice. Two weeks after 43 mice (female, strain A, 4 mos.) received 0.5 percent C (pH5) in their drinking water, they were administered 1 mg/g mouse urethan i.p. along with 82 controls. All the animals were sacrificed after 11 weeks and pulmonary adenomas counted visually. The average number of tumors per mouse was: controls, 7.77 ± 0.39; C treated, 5.17 ± 0.48. The observed difference and P values for controls vs. C treated were, 2.60, $P<0.00005$. All mice remained healthy and there were no observable abnormalities on autopsy.

2. In 1967, Walker et al. (8966) attempted to determine whether C protected mice against influenza A virus. White mice (16) were administered 300 mg/kg C i.p. Controls (16) were given saline. The results are shown in Table 14.

Table 12. Minimal Infectious Dose of Virus for Tissue Cultures Maintained in Medium Containing Ascorbic Acid (8966)

Virus	Infectious Dilution (TCD_{50}) in Titrations Performed in Cultures Treated with	
	Ascorbic Acid	No Ascorbic Acid
Rhino M	10^{-4}	10^{-3}
" H (16/60)	10^{-5}	10^{-5}
" H (FBB)	10^{-4}	10^{-4}
Polio 1	$10^{-5.5}$	10^{-5}
E.C.H.O. 11	$10^{-5.5}$	10^{-6}
Coxsackie A21	10^{-4}	10^{-4}
Influenza B	10^{-6}	10^{-6}
Parainfluenza 3	10^{-4}	$10^{-4.5}$
Respiratory syncytial	10^{-2}	$10^{-1.5}$
Adeno 5	10^{-2}	$10^{-1.5}$
Herpes	10^{-5}	10^{-5}

3. In 1969 Benade et al. (0695) showed that C is highly toxic or lethal to Ehrlich ascites carcinoma cells. The toxicity of C to these carcinoma cells was increased synergistically by concomitant administration of 3-amino-1,2,4-triazole (ATA). For experimental details see original paper. Experimental results showing the inhibition of respiration and cytotoxic action of ascorbate as well as the enhanced effect from combination with ATA are given in Table 13.

The authors concluded that the cytotoxic effect of ascorbate is due to the intracellular production of hydrogen peroxide. The enhancement of the C effect by ATA was shown to be due to its inhibition of cellular catalase, thus inhibiting the ability of the cells to detoxify hydrogen peroxide.

Table 13. Synergistic Cytotoxicity of Ascorbate and ATA (0695)

Addition	—	ATA 3 mg/cm³	C[1] 3 mg/cm³	ATA + C
Q_{O_2} 1st h	7.3	7.3	7.5	4.2
2nd h	7.5	7.4	5.3	2.8
3rd h	7.5	7.4	1.8	0.7
Final pH	7.28	7.30	7.28	7.25
Cell staining	trace (0.2%)	trace	50%	>95%

[1] C = Vitamin C (as Na-ascorbate)

14. In 1968, Wilson (9142) referred to data on the prophylactic effectiveness of C against the common cold and questioned whether C was remedying a dietary deficiency or acting as an anti-infective drug.

15. In 1968, Regnier (6846) discussing prevention and treatment of the common cold, emphasized the number of infective agents involved at any one time, the complex symptomatology, and the absence of criteria present in single infections. The author then discussed critically the symptomatology of scurvy, the knowledge of the actions of C, and a British trial of C against the common cold in which five of the known agents were isolated, and C was found to be without specific effects on any one of those agents, or on the subject who was challenged with concentrated amounts of those agents singly. He contrasted the positive results of the Dublin field trials with the foregoing negative conclusions, and concluded that the explanation was likely to lie in "a general vitamin deficiency" rather than in specific antigenic properties.

16. In 1969, O'Leary et al. (6096) reported a study on the effects of C supplementation on tooth mobility. The 17 subjects (21 to 55 years old) used in the 12-week study were divided into four groups:

Group I (4 subjects) received a placebo.

Group II (5 subjects) received a placebo for the first 6 weeks then 300 mg C daily for 6 weeks.

Group III (4 subjects) reversed the procedure with Group II.

Group IV (4 subjects) received C.

The mobility of six teeth was assessed for each subject before and at intervals during the experiment. The C supplement was found to have no significant ($P<0.05$) effect on tooth mobility.

17. In 1969, Wilson and Loh (9148) reported a distinction between toxic and catarrhal cold symptoms in 103 female students at boarding school:

(1) Toxic: sore throat, headache, feverish, out-of-sorts.

(2) Catarrhal: head-cold, cough, nasal obstruction, nasal discharge.

Symptoms within these groups were associated, but the two groups were unrelated to each other. Students received either 200 mg/day of C or a placebo. The C, but not the placebo, raised leucocyte C levels by nearly one-half, reduced the incidence, duration and severity of both types of cold, and reduced

The author concluded that significant benefit was obtained by the C therapy in disc treatment and that the postoperative course in most cases was improved, recurrences were reduced and greater confidence in the operation could be anticipated.

11. In 1964, Voute (8906) observed that a condition of "disorientation" occurring with some primiparae was amenable to C therapy. One serious case and two mild cases were described as quickly and completely cured with C therapy.

12. In 1966, Walker et al. (8966) attempted to demonstrate that C protected man against cold producing viruses. For three days before receiving intranasal drops containing a small dose of virus (an M rhinovirus, an H rhinovirus, a "new" rhinovirus which grows only in organ cultures, influenza virus type B, or a "new" virus related to avian bronchitis), 91 volunteers received 3 g C daily p.o. (75 mg/kg/day).

The number of volunteers developing colds is shown in Table 26. It indicates that the C had no effect on the number of colds and their severity. The results of virus isolation studies and of antibody filtrations on acute and convalescent sera is shown in Table 27. These results show that the treatment with C had no effect on the frequency of virus infection.

The authors concluded that there was no evidence that the administration of C prevented colds produced by given known viruses. Some previous experiments supporting the view that C may reduce the duration of pharyngitis or colds is summarized in Table 28.

13. In 1967, Wilson (9141) commented about the Walker et al. study (see this above) in a letter to the British Medical Journal in which he questioned the interpretation of their results.

(1) The sexes and ages of their selected group of volunteers were not given.

(2) The number of inoculated subjects was small (91), and no information was given about whether all had received comparable C doses prior to the investigation.

(3) The method of infection was artificial.

The results of the author's own large field surveys in Dublin (see this section p.50) do not appear to support the totally negative view of the therapeutic effect of C arising from the Walker et al. study.

8. In 1963, Milner (5587) described the results of a controlled blind study in which 40 male chronic psychiatric patients were given either 1 g C daily for three weeks or a placebo. Some of the patients were initially found to show signs of scurvy which disappeared with C administration.

The author noted that:

(1) Psychiatric patients have an unusually high demand for C.

(2) A statistically significant improvement was seen following C saturation in depressive, manic and paranoid symptoms along with improvement in overall personality functioning.

The author suggested that chronic psychiatric patients would benefit from C administration.

9. In 1963, Schmidt et al. (7418) surveyed earlier findings on the relation between C and tumors in man and found many differences among them, e.g.:

(a) Increased tolerance to x-ray irradiation and inhibition of cancer growth and found by both Schneider and Wendt (references in original paper) when 2000 mg C per day was given.

(b) Another researcher (reference in original paper) reported observing a better response to several weeks of irradiation with patients on a C-free diet, than with controls.

(c) Huber (reference in original paper) recorded 25 serious gynecological cancer cases who showed a general improvement with 2000 mg C/day but no change in the tumors.

(d) Rheinhold (reference in original paper) also reported an improved general condition but with some partial tumor regression in 218, mostly inoperable, cancer cases given additional C.

(e) Other authors (references in original paper) reported generally favorable effects of C on the blood in anemia, leukemia and irradiation.

10. In 1964, Greenwood (3180) reported favorable results in patients having disc lesions treated with therapeutic doses of C. Over 500 patients (including the author) were treated.

Patients with early disc lesions as well as those with either simple lumbosacral strain or definite sciatic nerve involvement received 750 to 1000 mg C daily. Presurgical cases were also given C therapy and a small percentage recovered (with some later recurring). Patients with chronic generalized disc involvement and postoperative patients were also put on similar therapy.

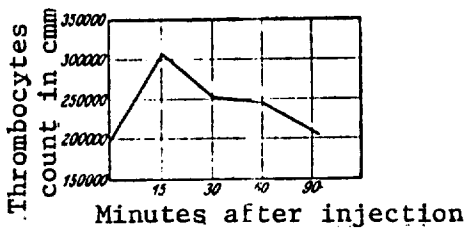

Figure 5. Effect of a Single Injection of Vitamin C (500 mg) on the Thrombocyte Count (6268)

4. In 1939, Telegdi (8353) suggested that patients with typhoid fever might have a hypovitaminosis C and would thus benefit from C therapy. He found that C requirement was directly related to the severity of the case and that during treatment with C there was a reduction in toxic symptoms accompanied by an increase in thrombocyte count. (See this section I,3 for a similar finding.)

5. In 1941, Campbell(1319)reported the successful treatment of 14 cases of gingivitis with large C doses. The patients were given 300 mg C/day until saturated as indicated by the presence of 5 mg per 100 ml urine. The total C needed to produce saturation varied from 900 to 4200 mg. A noticeable improvement was seen after 300 mg. The gums returned to normal after about four days treatment (2000 mg C, average needed). A maintenance dose was recommended.

6. In 1944, Pelner (6368) reported that C protected an extremely sensitive ragweed patient against adverse reactions to pollen injections. The author also cited the published observations of Holmes (reference in original paper) that 500 mg C/day relieved hay fever patients and that C was found to protect against food allergies.

7. In 1950, Massell et al. (5311) reported on their observations suggesting that large doses (4 g/day) of C possess antirheumatic activity. The report deals with seven patients with rheumatic fever administered C for eight to 26 days. The authors considered their results sufficiently worthwhile to warrant further study particularly with respect to the relation of C to the activity of the adrenal cortex.

Table 25. Effects of C on Thrombocytes in Patients with Various Diseases (Cont'd)

No.	Diagnosis	Vitamin C-dose	Thrombocytes in ccm, according to Fonio	Thrombocytes in ccm, according to Flossner	Time of Determination
4	Hypophyseal cachexia	3500 mg (daily 500 mg)	100000 405000		a c
5	Diabetes mellitus	2000 mg (daily 500 mg)	290000 450000	602000 900000	a d
6	Diabetes mellitus	1500 mg (daily 500 mg)	170000 332000		a c
7	Croupous pneumonia	6000 mg (daily 1000 mg)	255000 530000 480000	650000 1300000 1355000	a b d
8	Influenzal pneumonia	1500 mg (daily 500 mg)	250000 370000	 1000000	a b
9	Diabetes mellitus	100 mg	195000 200000		a b
10	Fracture of the neck of the femur	1500 mg (daily 500 mg)	272000 360000	674000 935000	a c
11	Chronic polyarthritis	900 mg (daily 300 mg)	290000 325000 300000		a b e
12	Heart failure	750 mg (daily 250 mg)	220000 215000 250000		a b e
13	Lobar pneumonia	1000 mg (daily 500 mg)	260000 302000	700000 750000	a c
14	Pulmonary tuberculosis	1500 mg (daily 500 mg)	150000 270000		a b
15	Chronic polyarthritis	500 mg (daily 100 mg)	197000 184000 190000		a b e
16	Pleuritis carcinomatosa	100 mg	140000 132000		a b
17	Chronic bronchitis	400 mg (daily 100 mg)	168000 291000		a b
18	Werlhef's disease*	7000 mg (daily 500 mg)	8000 62000 70000 70000		a j f i
19	Lobar pneumonia	2500 mg (daily 500 mg)	220000 465000 200000		a b f
20	Gastric (peptic) ulcer	4000 mg (daily 500 mg)	150000 460000 390000		a b e

Table 33. Clinical features of scurvy appearing between day 26 and day 180 in human subjects on scorbutogenic diets. Descriptions of the features are derived from four studies in which experimental scurvy was produced by administration of vitamin C-free diets for up to 180 days, and from five accounts of clinical scurvy in Britain in which the diagnosis was confirmed by regression of lesions after administration of vitamin C. (References are obtainable from the author.) (9146)

Capillary and vascular changes Petechial tongue lesions Petechial haemorrhages Conjunctival haemorrhages Bleeding from the gums Nose-bleeds Ecchymoses Bruises Haematuria	*Respiratory changes* Dyspnoea Tonsillitis Tenderness of buccal mucosa Dental caries Xerostomia Colds
Changes in the skin Coiled hairs Congested follicles Loss of hair Acne Hyperkeratosis Scleroderma Pruritus Skin scaling Pigmentation: brown or purple Sjögren's syndrome	*Changes in peripheral nerves* Femoral neuropathy Skin ulceration
Changes in joints, bones and muscles Arthralgia Joint effusions Bone tenderness Aching of limbs Pain and discoloration of legs	*Mental changes* Physical fatigue Subjective fatigue Lassitude Drowsiness and lethargy Depression Hypochondriasis Hysteria and anxiety
	Metabolic changes No change in appetite Weight loss Oedema

27. In 1973, Wilson (9144) reported the results of a double-blind clinical test with the students in two male and two female boarding schools who were given either dummy or 200 mg tablets of C or 200 or 500 mg tablets of C daily. Examination of blood levels revealed that the girls reached higher blood levels than the boys and utilized the vitamin differently. They found that the girls received more beneficial effects with respect to reduction in severity, intensity and complexity of colds than the boys.

The author concluded that his results suggest that 2000 mg daily is the required dose for a beneficial prophylactic effect against the common cold in about 90% of adult females and that adult males require 2500 mg daily to achieve the same effect. He noted that the innate ability of females to maintain tissue saturation with C when under stress more efficiently than males is a type of sex differential which has not previously been shown to be characteristic of a vitamin.

28. In 1974 Kakar and Wilson (4084) reported that plasma and leucocyte C levels were reduced in children with acute lymphatic leukemia, and in an elderly patient with lung cancer. The C level in skin metastases in the latter case was higher than in normal skin. The authors suggested that growing tumor tissue in humans selectively concentrated C.

29. In 1974, Wilson (9146) tabulated some effects of scurvy as in Table 33.

30. In 1974, Loh et al. (5007) reported that leucocytes from subjects with atropic allergy took up, in vitro, significantly ($P<0.001$) less C in the presence of a specific antigen, whereas leucocytes from control subjects showed no difference in C uptake when antigen was added. The authors suggested that leucocyte uptake of C under these conditions might be a useful test for the presence of atopic allergy. One of the authors has also suggested (9145) that these findings, at a pilot-study level, show that leucocyte saturation with C improves the immunological mechanisms.

26. In 1973, Briggs and Briggs (1118) examined the effects of C supplementation on leucocyte and plasma ascorbate in untreated healthy women, geriatric patients and women under treatment with various steroid hormones.

The results summarized in Table 32 show that:

(1) Leucocyte and plasma ascorbate levels were found to be low in (a) geriatric patients (b) menopausal women taking estrogen and (c) young women taking combined-type oral contraceptives.

(2) Untreated controls and young women either taking a progestagen-only contraceptive or long-acting progestagen injections had similar ascorbate levels.

(3) C supplements increased leucocyte ascorbate in all groups; (a) 25 to 45 percent in controls and progestagen-treated young women; (b) 95 to 135 percent in geriatric patients and estrogen-treated women.

(4) Larger, similar increased plasma ascorbate was also found (a) 75 to 90 percent in controls and progestagen-treated women; (b) 125 to 235 percent in geriatric patients and estrogen-treated women.

The authors postulated that estrogens increased ascorbate breakdown lowering tissue levels possibly by stimulating release of ascorbate from such cells as leucocytes into plasma.

They concluded that the C requirements for estrogen-treated women are probably therefore increased.

Table 32. Leucocyte and Plasma Ascorbate in Women (1118)

Group	No.	Mean age (yr.)	Condition	Ascorbate concentration (mean ± S.D.)			
				Leucocytes (μg. 10^7)		Plasma (mg./100 ml.)	
				Before	After (a)	Before	After (a)
A	16	23	Healthy, untreated	35 ± 12	48 ± 15	0·9 ± 0·19	1·6 ± 0·30
B	19	25	Healthy, combined oral contraceptives (b)	24 ± 16	46 ± 19	0·4 ± 0·30	0·9 ± 0·41
C	16	22	Healthy, progestagen-only oral contraceptive (c)	36 ± 11	51 ± 17	0·9 ± 0·22	1·7 ± 0·39
D	12	30	Healthy, depot progestagen (d)	38 ± 17	48 ± 16	0·8 ± 0·21	1·5 ± 0·32
E	10	68	Geriatric patients	23 ± 11	51 ± 14	0·5 ± 0·16	1·5 ± 0·30
F	10	56	Menopausal, œstrogen therapy (e)	20 ± 9	47 ± 16	0·3 ± 0·32	1·0 ± 0·40

(a) After 500 mg. daily vitamin C for 14 days.
(b) 50 μg. daily ethinylœstradiol plus either 1·0 mg. daily norethisterone acetate or 0·25 mg. daily *d*(−)-norgestrel.
(c) 0·35 mg. daily norethisterone.
(d) 150 mg. 3-monthly medroxyprogesterone acetate i.m.
(e) 625 mg. daily conjugated œstrogens.

25. In the same year Anderson et al. (0190) reported the results of their double-blind trial in which 818 subjects participated, 411 on a placebo and 407 taking 1000 mg C/day for three to four months. The overall sickness experience of the two groups is summarized in Table 31 in terms of frequency of episodes, duration of symptoms and duration of "disability" (as measured by number of days confined to the house).

It was found that:

(1) The C group was ill fewer days than the placebo group.

(2) There was a statistically significant difference ($P<0.05$) between the two groups with respect to the number of subjects who remained well throughout the experiment.

(3) The C group had 30 percent fewer days when they were confined to the house or unable to work than the placebo group ($P<0.0001$).

(4) The C group also had a lower incidence of chills and severe malaise, the symptoms of acute illness.

The authors concluded that their unexpected finding that disability was substantially less in the vitamin group could have important theoretical and practical implications.

Table 31. Overall Sickness Experience of the Subjects in the Vitamin and Placebo Groups (0190)

	Episodes of illness		Days			
			Symptoms present		Confined to house	
	V	P	V	P	V	P
Total number	561	609	2138	2474	531	769
Number per subject: Mean ± S. E.	1.38 ± 0.061	1.48 ± 0.056	5.25 ± 0.297	6.02 ± 0.284	1.30 ± 0.101	1.87 ± 0.138
t—value	1.21		1.87		3.33**	
V/P	93%		87%		70%	
†Mean number per episode: Mean ± S. E.	—		3.96 ± 0.162	4.18 ± 0.139	1.04 ± 0.074	1.32 ± 0.087
t—value	—		1.03		2.45*	
V/P	—		95%		79%	

V = vitamin group, P = placebo group
Approximate statistical probabilities: * < .05, ** < .001
†These figures are based on the average values for each subject. The corresponding mean values calculated direc ly from the over-all number of days and episodes were: (symptoms) 3.81 and 4.06, (house) .95 and 1.26.

review led them to speculate that individuals with low serum ascorbate levels are the ones with the highest probability of forming bladder tumors. They concluded that the ingestion of C should be encouraged in persons who, owing to age, cigarette smoking, or other factors may be prone to bladder tumor formation.

23. In 1971 Pauling (6328) calculated the combined statistical significance of four published studies by others, as:

(1) Effect on incidence of common colds was significant at the 98.86% level of confidence.

(2) Effect on integrated morbidity of colds was significant at the 99.9978% level of confidence.

The studies were by Ritzel, Cowan, Diehl and Baker, Wilson and Loh, and Franz, Sands and Heyl.

24. In 1972, Charleston and Clegg (1442) reported the results of a survey carried out between November and March 1971 with 47 adults, to ascertain the effect of 1 g daily doses of C on the incidence and severity of the common cold. The results are summarized in Table 30.

The authors concluded that on the basis of a one-tailed statistical test, 1 g C per day was effective in reducing cold incidence at the 0.002 level of significance. The duration was less at the 0.05 level of significance. The authors pointed out that their survey had found that at the 1 g C/day supplementation level, the reduction in the incidence of colds was more significant than the reduction in duration of cold symptoms.

Table 30. Incidence and Duration of Colds During 15 Weeks in Winter (1442)

	Ascorbic acid 1 g./day	Placebo
No. of persons in group	47	43
No. of persons having: 0 cold(s)	16	6
1 „	19	11
2 „	11	14
3 „	1	7
4 „	0	5
Total no. of colds	44	80
Average no. of colds per person	0·94	1·86
No. of colds of: 2 days' duration	12	2
3 „ „	13	11
4 „ „	12	38
5 „ „	5	26
6 „ „	1	3
14 „ „	1	0
Average duration of cold (days)	3·5	4·2

the symptom-association within each type of cold by influencing some symptoms more than others.

18. In 1969, Hindson and Worsley (3591) found that 1 g C daily reduced the prickly heat and hypohidrosis induced in men by polythene occlusion.

19. In 1969, Linner (4957) examined the effect of C therapy on ocular hypertension. A group of 25 patients (63 years average age) with moderate ocular hypertension was given C, 0.5 g four times daily for six days p.o. A significant fall in the intraocular pressure was observed but no significant change in the facility of outflow.

20. In 1970, Gey et al. (2820) investigated whether supplemental doses of C would improve endurance performance and reduce the severity and morbidity of athletic injuries. The experimental subjects (286, average age 28 years) received either a placebo or 1000 mg C daily for 12 weeks. The results of the endurance test are summarized in Table 29.

The authors concluded that:

(1) The effect of C on endurance was negligible.

(2) The percentage of injuries in both C and placebo groups was comparable.

Table 29. Performance on 12-Minute, Walk-Run Test (2820)

		At Beginning of Training		After 12 Weeks of Training	
Pills Taken		No. of Subjects	Distance (Miles)	No. of Subjects*	Distance (Miles)
All	Ascorbic acid	112	1.32 ± 0.23	111	1.65 ± 0.16
	Placebo	100	1.30 ± 0.24	96	1.62 ± 0.17
Less than one half	Ascorbic acid	29	1.20 ± 0.22	27	1.60 ± 0.15
	Placebo	45	1.34 ± 0.26	44	1.62 ± 0.17
Total	Ascorbic acid	141	1.30 ± 0.23	138	1.65 ± 0.16
	Placebo	145	1.30 ± 0.24	140	1.62 ± 0.17

*Five subjects were unable to take the final running test because of athletic injuries.

21. In 1970, Sullivan and Eisenstein (8184) reported that C is removed from the plasma of patients undergoing hemodialysis. They therefore recommended dietary C supplementation for such patients.

22. In 1970 Schlegel et al. (7401) discussed the significance for humans of their experimental results in preventing bladder tumor formation in mice with 3-HOA pellet implants, by C administration. (See this section B,3 p.000). They suggested that oral C administration might prevent spontaneous bladder tumor formation in humans. They noted that cigarette smoking significantly lowers both the plasma and leucocyte C concentration which apparently leads to chronic disease both cancerous and non-cancerous. They reported that a literature

4. Regulation of C in the United States

In CFR Title 9 (0258) Section 318.7, ascorbic acid is described as a curing agent, to accelerate or preserve coloration in cured pork and beef cuts and comminuted meat products. The specified amount is 75 oz/100 gall. pickle at 10% pump level, 0.75 oz/100 lbs meat or meat by-product, 10% solution to surface of cured cuts before packaging but this must not add significant amounts of moisture.

In Section 319.105 ascorbic acid can be added to "chopped ham" in amounts not over those mentioned above. In Section 381.147 similar provisions are made for poultry and poultry products.

In CFR Title 21 (0259) the compound D-erythroascorbic acid with its synonyms including D-isoascorbic acid was changed (Section 3.51) to erythorbic acid (common name) to avoid misleading confusion with ascorbic acid, because erythorbic acid has no C-activity, and is not specified as an ingredient of any food for which standards have been established.

In Section 121.101 ascorbic acid is listed as GRAS without data on tolerance, or limitations or restrictions. It is classed as a chemical preservative, and a nutrient and/or dietary supplemtnt.

In Section 125.3 claims made for a food because of its C content must be supported by specific information on the label, and the minimum daily requirements are defined as: 10 mg for an infant, 20 mg for a child, and 30 mg for an adult (1 mg also = 20 USP units).

Section 125.5 states that infant foods should contain at least 7.8 mg of C/100 kcal, and should be labeled accordingly if this has to be made up from "other sources."

5. Official Exposure Data

Ascorbic Acid

Table 56. Import Poundage (8611)

"Vitamin C, or Ascorbic Acid and Its Salts (In pounds)

	General Imports	Imports for Consumption
1972 (Jan.-Nov.)	4,998,474	5,014,045
1973 (Jan.-Nov.)	5,333,444	5,293,174

2. FAO/WHO

In 1962, the FAO/WHO (4009) stated that ascorbic acid was used as an antioxidant in emulsions of fats and oils and as a browning inhibitor in unprocessed cut fruits fruit pulps and juices.

In 1966, the FAO/WHO (4010) stated that "acceptable daily intakes for man" of C were 0 - 2.5 mg/kg unconditionally, and 2.5 - 7.5 mg/kg conditionally on expert supervision.

In 1970, the FAO/WHO (4011) listed the functions of C as coenzymatic, in wound healing, infections and stresses, in vascular reactivity, amino acid metabolism, hemostasis and blood-clotting; however, "there is no reliable evidence that large doses of ascorbic acid protect against infections such as the common cold, or that excessive amounts of the vitamin are utilized during severe infectious diseases". The report emphasized that low plasma levels of C "do not necessarily indicate true deficiency", and stated that saturation tests "do not provide quantitative information on requirements".

On requirements, the same report (4011) accepted that "slightly less than 10 mg daily" was the minimum needed to prevent or cure scurvy, that scurvy developed after 90 days of total deficiency, and that leucocyte C levels were better indicators than plasma C levels. Daily intakes of 10 - 22 mg had maintained leucocyte C levels for up to 90 days. Recommended daily intakes were: birth to 12 years, 20 mg/day; adolescents and adults 30 mg/day; and pregnant or lactating women 50 mg/day. The list of references omits large sections of the experimental evidence referred to in this monograph, and (for example) cites Bourne's work of 1946, omitting his conclusions of 1949 (1037).

3. Other

In the U. S. Dispensatory (6184) the oral therapeutic dose of C is given as 500 mg/day. The optimal daily nutritional requirements are given as 75 mg for a 65 kg man, 70 mg for a 55 kg woman, 100 mg during pregnancy, 150 mg during lactation, infants 30 mg, children 1 - 3 years 35 mg, 4 - 6 years 50 mg, 7 - 9 years 60 mg, 10 - 12 years 75 mg, girls 13 - 20 years 80 mg, and boys 13 - 20 years 90 - 100 mg.

from scurvy, with a "safe margin", on maintenance of specific functions including wound healing, metabolism, resistance to specific stresses (but not including common colds), excretion data, and comparative nutrition of various animal species.

Table 55.

Leucocyte Ascorbic Acid Concentrations. Values Related to Age in Healthy Control Subjects, in Smokers, and Related to Petechiae and Purpura in the Elderly. Values Four Hours After a Loading Dose of 500 Mg of Vitamin C During and Following Common Cold, and Mean Value During and 10 Days After Onset of Cold Symptoms. Reported Values in Gastrointestinal Disorders. (9146)

State of health	Leucocyte concentrations (Mean and standard deviations)		Reference
	Male	Female	
Healthy controls (age in years)			
4 to 12	56·4 ± 21·9	—	Loh and Wilson, 1971
11 to 18	36·6 ± 9·1	42·8 ± 12·6	
22 to 49	30·4 ± 8·7	34·0 ± 10·9	
56 to 87	24·4 ± 13·6	23·4 ± 11·3	
Smoking			
Non-smokers	24·6 ± 1·3	30·7 ± 1·4	Brook and Grimshaw, 1968
Moderate	19·6 ± 1·7	25·6 ± 1·6	
Heavy	17·4 ± 3·5	27·8 ± 1·4	
Petechiae and purpura in aged			
Sublingual petechiae (range and mean)	5·1 – 21·3	10·23	Andrews and Brook, 1966
Senile purpura	6·4 – 31·7	16·0	
Positive Hess test	15·2 ± 1·2	-27·9 ± 2·3	Eddy, 1972
Common cold			
During cold—3rd day	24·7 ± 9·7	27·6 ± 5·8	Wilson and Loh, 1974
3 weeks post-cold	32·9 ± 8·6	34·1 ± 11·6	
During cold—1st day	10·3 ± 0·3		Hume and Weyers, 1973
10 days after onset	24·0 ± 6·5		
Peptic ulceration			
Controls	17·9 ± 0·9		Russell *et al.*, 1968
Gastrointestinal haemorrhage	14·2 ± 0·9		
Aspirin or alcohol ingestion	12·6 ± 0·9		
Without history	18·2 ± 0·8		
Duodenal ulceration			
Controls	28·7 ± 8·1		Dymock *et al.*, 1968
Stenosis	11·7 ± 4·5		
Gastric surgery:			
without symptoms	10·1 ± 4·5		Cohen and Duncan, 1967
with symptoms	20·4 ± 8·4		
Duodenal ulcer:			
before surgery	8·7 ± 3·1		
after surgery	12·8 ± 4·3		
Controls	22·9 ± 5·9		
Duodenal ulcer general	10·6 ± 4·9		Esposito and Valentine, 1968
Following gastric surgery	12·7 ± 6·8		Williamson *et al.*, 1967
Controls	22·1 ± 6·4		Esposito and Valentine, 1968
Gastrointestinal disease			
Gastroduodenal disorders	11·0 ± 4·4		Cohen and Duncan, 1967
Intestinal malabsorption	10·7 ± 5·2		Williamson *et al.*, 1967
Controls	20·8 ± 10·5		

In 1971, Spitznagel (7990) calculated that the studies indicating efficacy of C at doses of 1 - 5 g/day against the common cold fitted a lognormal distribution with large variance. However, certain other studies did not fit this distribution, and the author suspected the presence of extraneous variables in these studies. He urged further statistical studies of this approach for estimating human requirements of C.

In 1971, Wilson (9143), reviewing six years work in relation to Pauling's expressed opinions, stated there were two opposite views about human C requirements. One held that only enough C was needed to prevent scurvy; the other that humans should be saturated as were animals that made their own C. The author emphasized the need for controls in clinical trials of C against the common cold to identify:

1. Sex and age influences on C metabolism, which he stated were large,
2. Tissue integrity, which he stated was greatly influenced by initial C status.

The author emphasized the need for more data on C status of individuals with colds compared with status at other times. He criticized Pauling for not providing such information to support his thesis of unlimited therapeutic efficacy for C against common cold symptoms.

In 1971, Manchanda et al. (5195) measured the plasma C levels of 100 healthy and 400 sick children (age range under 6 months to over 5 years) with malnutrition infections, or gastrointestinal disorders. Two-thirds of each group had plasma C levels 0.5 - 0.7 mg/100 ml and were regarded as consuming less than their requirements of C.

In 1972, Sim (7760) emphasized that daily human requirements for C were not agreed; estimated mean intakes concealed large seasonal fluctuations, so that many people must become seasonally deficient. But scurvy took long to become overt.

In 1974, Wilson (9146) compared the RDA's for C in the nations shown in Figure 18. He attributed the wide range to the operation of two different concepts. On the one hand, "the negative concept in which the desirable dose is that which prevents deficiency"; on the other, "the positive concept that vitamin C can improve tissue efficiency and integrity when it is available in sufficient quantity", defined as "necessary for normal metabolic function". This amount is stated to vary between individuals and according to the condition of the individual because of desaturation by many pathological conditions (Table 55).

Table 53.

Average Daily Intake of Nutrients During a Survey Period of One week. The Daily Intake of Protein, Fat and Carbohydrate is Measured in Grams, That of Calcium, Iron and Vitamin C in Milligrams and the Energy Intake is Measured in Kilocalories. The Average Intake of the Dublin Sample is Indicated as a Percentage of the Recommended Dietary Allowance in Great Britain. (9147)

	Aged 70 and Below	% Recommended Intake	Aged Over 70	% Recommended Intake	% Change With Age
Protein	59.1	116	52.5	109	-11
Fat	76.9	---	72.3	---	-6
Carbohydrates	192.8	---	198.6	---	+2
Calories	161.9	79	158.2	83	-3
Calcium	391.0	78	475.0	95	+21
Iron	16.5	165	14.5	145	-12
Vitamin C	34.0	113	31.0	103	-9

Table 54.

Range of Daily Intake of Vitamin C, Calcium and Iron In Milligrams in the Dublin Sample Compared with the Recommended Intake in Great Britain. (9147)

Nutrient	Recommended Daily Intake	Simple Mean Intake	Range of Intake	% Less Than Recommended Intake
Vitamin C	30	32	18 - 56	36
Calcium	500	471	204 - 793	61
Iron	10	148	30 - 234	6

In 1971, Hodges et al. (3624) studied five healthy male prisoners in a metabolic ward and found that the full syndrome of scurvy did not appear until normal body stores were depleted below 300 mg, and that under 10 mg/day would prevent or cure scurvy. They concluded that optimal intakes should be estimated on their data "plus a knowledge of the biological and physiological variables of mankind."

In 1963, McDonald (5400) found in 282 Navajo students with mean C intakes of 69 mg/day of C, that 10% had gingivitis related to C deficiency. However, they admitted that losses of C in storage and preparation of foods might have exaggerated the reported intakes.

In 1965, Guggenheim and Margulec (3251) found that 115 old people at Jerusalem, economically poor, had intakes from vegetables of 47 - 51 mg of C/day without signs of nutritional deficiency.

In 1966, Winter et al. (9162) found in Haifa that the C content of human breast milk averaged 6.94 mg% in May and 4.90 mg% in November. Infants receiving cows' milk plus fruit juice were not significantly different in C status from those receiving breast milk alone, and the latter were judged not to need supplementary C.

In 1969, Tattersall, reviewing the history of requirements for C, noted that the lemon juice provided to British merchant sailors in 1894 would have supplied C at 10 mg/day, but the lime juice only at 4 - 5 mg/day. In 1927, concentrated orange juice was authorized at 0.5 fl. oz/head/day, supplying 30 - 50 mg/day of C. In 1933, the League of Nations Technical Commission proposed 30 mg/day for an adult. In 1950, the British Medical Association recommended 20 mg/day for adults, 30 mg/day for adolescents, and 50 mg/day for lactating women. In 1953, the Medical Research Council Report compiled by Bartley, Krebs and O'Brien found that scurvy took 17 weeks to appear in deprived control subjects and 7 - 9 weeks to disappear when 10 mg/day was given. Subjects given 70 mg/day were fully healthy. The British RDA was thereupon set at 30 mg/day.

In 1970, Pauling, in a book (6329), expressed the opinion "that for most people the optimum daily intake is somewhere between 250 mg and 10 g." He criticized the RDA published by NAS NRC as too narrowly based, citing statements that 10 mg/day was enought to prevent scurvy, that the RDA of 35 - 60 mg/day (infants to adult males) was, therefore, "generous", and that male physical and psychomotor performances had not been improved by up to 300 mg/day. Pauling considered that the NAS NRC had failed to take into account tissue requirements for C for other functions including membrane maintenance, and the normal provision of 100 - 300 mg/day from "a good ordinary diet".

In 1970, Wilson and Nolan reported a survey of the diets of 28 women and 3 men (aged 64 - 86) in Dublin, Eire (Tables 53 and 54).

In 1948, Hsu et al. (9461) found that Chinese college students required 89 mg of C/day for tissue saturation.

In 1949, Sigurjonsson (7743) surveyed dietary C intake in Iceland and found that it averaged about 34 mg/day from 1936 to 1945, with a probable seasonal variation of 20 - 50 mg/day. The major sources were potatoes and milk, and the population "as a whole" showed no obvious signs of scurvy, but "subclinical" scurvy "may occur occasionally". The authors concluded that the League of Nations recommendation of 30 mg/day for adults was "fully adequate".

In 1949, Bourne (1037) reviewed the known relationship of C to immunity, defined as resistance to infections. He concluded that the evidence was not consistent but the weight of it was against any role for C in maintaining the complement titer of the blood. The action of C in enhancing production of scar tissue might explain beneficial reports in tuberculosis. Disparities in evidence on the inactivation of toxins including diphtheria toxin might be explained by variables in the reported experiments, such as pH, incubation time, or media differences. Deficiency of C was reflected in leucocytes and by depression of their activities, particularly phagocytosis.

The author concluded by comparing human requirements for C with his estimate of that of the gorilla. On the basis that the gorilla utilized 4000 - 5000 kcal/day from green feed, i.e., 20 lbs of green feed, this would provide about 4.5 g/day of C. Therefore, "when we are arguing whether 7 or 30 mg of vitamin C a day is an adequate intake we may be very wide of the mark. Perhaps, we should be arguing whether 1 or 2 g a day is the correct amount. Perhaps it is normal for our blood and tissues always to be saturated with the vitamin and ... that continuous doses of vitamin C at this level over a considerable period of time may have a pronounced and unequivocal anti-infective action."

In 1957, Martin et al. (5271) reported intakes and serum levels of C in 2129 pregnant women. Levels decreased during pregnancy, again after delivery, and were lower if the mother lactated. Intakes of 80 - 100 mg/day maintained serum levels during pregnancy at average 0.7 mg%, but with 120 mg/day the serum of lactating women did not exceed 0.3 mg%. The authors concluded that C status was unimportant except in five conditions where it was "at most a contributory factor" because the relation between intake and serum level was never strong.

They also stated: "The daily requirements of an adult are considered to be about 25 mg. for the prevention of scurvy and more than that for normal requirements."

In 1939, Snelling (7898) estimated plasma C levels in 158 infants and 77 older children, finding in infants 0.76% mg% when breast-fed, and 0.12 - 0.30 mg% when formula-fed, but only 3 of 58 with under 0.10 mg% were scorbutic. In the children (aged 2 - 14) values averaged 0.76 mg%, with lower values in respiratory infections. Administration of 100 - 1000 mg C in one dose raised some plasma levels, but most subjects required daily amounts and the author concluded that the plasma values were no guide to C status.

In 1939, Hoygaard and Rasmussen (3760) reported that the Angmagssalik Eskimos received about 40 mg/day of C, half from narwhal and half from animal and fish sources. Scurvy was unknown.

In 1939, Goldsmith and Ellinger (3049) found a relationship between plasma and urinary C levels, and concluded: "Mild vitamin C deficiency is extremely prevalent, and too much importance should not be attached to depletion of the ascorbic acid content of the body in any disease."

In 1941, Rafsky and Newman (6719) gave 25 normal subjects (aged 66 - 88) 200 - 1000 mg/day of C in order to assess requirements in old age. They reported retentions that were higher than expected, and concluded that geriatric requirements should receive further study.

In 1943, Roberts et al. (6973) studied the C requirements of 30 girls (aged 6 - 12) using blood levels of 0.7 mg% and 24-hour excretion of 50% of a 300 mg test dose as the criteria. The authors concluded that 62 - 72 mg/day "would seem to be an adequate allowance for pre-adolescent girls of 6 to 12 years."

In 1943, Purinton and Schuck (6684) studied the C requirements of 63 subjects by the i.v. saturation test dose method. They concluded that subjects aged less than 25 required over 100 mg/day, older subjects less, that less was required with lower basal metabolism rates, and that there were relationships between plasma C and hemoglobin levels, and between retention of C and excretion of citric acid.

In 1946, Lowry et al. (5041) found that human subjects who received 8 mg/day C for eight months were able to retain about 1800 mg of C when given large doses. They concluded that the normal adult store of C was about 4 g, and that leucocyte concentration was a "valid index". When the intake was 8 or 23 mg/day, the leucocytes contained about 12 mg% C; when intake was 78 mg/day, the leucocytes contained about 25 mg%.

In 1971, Greene et al. (3176) found that C in combination with propyl gallate or butylated hydroxyanisole prolonged the shelf-life of ground beef both by inspection criteria and by metmyoglobin levels; bacterial spoilage was not, in the authors' opinion, masked.

A number of the authors cited in this section (e.g., 5400) have emphasized the influence of storage and preparation on C content. Keeping a meal warm for an hour between preparation and consumption can lose half of the C content in some foods.

C. Special Effects (Guinea Pigs)

In 1971, Wagstaff and Street (8949) found in guinea pigs that higher intakes of C were required to maintain induction (by dieldrin) of liver hydroxylating enzyme systems, than were needed to prevent scurvy.

A very recent (1974) study by Thaete and Grim (8388) in guinea pigs has demonstrated electronmicroscopically that C enhances the formation of buccal cell membranes. The authors conclude: "For the maintenance of optimal physical integrity of buccal epithelial cells, ascorbic acid may be necessary in larger doses than the minimum required for the prevention of scurvy. It is proposed that doses up to 100 times the minimum may be beneficial."

D. Human Requirements (Reported in the Literature)

In 1936, Van Eekelen (8693) conducted a classic experiment on C requirements using herself as the subject. She concluded that the blood and urinary levels of C depended on the amount ingested and the amount stored in the body. Saturation coincided with a blood level of about 13 mg/liter, and the urine excretion test was judged the most reliable ancillary test. "The daily requirements are dependent upon the amount stored in the organism, they are largest when the subject does not become markedly unsaturated. The daily dose required for adults weighing 70 kg amounts to about 60 mg under normal conditions."

In 1938, Heinemann (3500) calculated that a subject with plasma C 0.8 mg% would require 1 g of C for saturation, with 0.4 mg% about 2 g, and that daily requirements were about 0.8 mg/kg. The amount that would protect against scurvy was estimated as 0.4 mg/kg.

In 1938, Abt and Farmer (0046) reviewed the pharmacology of C. They stated "Although the normal daily human requirements have been estimated to be from 15 to 40 mg. or even up to 60 mg., it is difficult to explain why certain therapeutic effects can be obtained only by administering doses of from 10 to 25 times this amount."

In 1922, Sherman, LaMer, and Campbell devised a guinea-pig bioassay for antiscorbutic activity (9024).

In 1928, Szent-Gyorgyi isolated crystalline hexuronic acid from oranges, cabbages, and adrenals (8336) and in 1930, Tillmans suggested that this was the vitamin (9407); in 1932 Tillmans, Hirsch, and Jackisch developed the indophenol chemical assay for it. In 1932, Zilva inferred that the vitamin was derived from hexose; King and Waugh isolated hexuronic acid from lemon juice and identified it with Szent-Gyorgyi's compound and Szent-Gyorgyi found hexuronic acid to be active in guinea-pigs and renamed it ascorbic acid (8336).

In 1933, ascorbic acid was synthesized by Reichstein in Switzerland and by Howarth in England (8336); in 1934, both workers confirmed its identity with hexuronic acid (9407), and Fish and Harris showed its involvement in tooth development (8336). In 1936, Hopkins and Morgan reported that reduction of C was reversible and was mediated by glutathione; in 1938, Szent-Gyorgyi concluded that C functioned in hydrogen transport and tissue oxidation, and he received a Nobel Prize (8336).

In 1952, Schwartz and Williams discovered the role of C in the conversion of folic to folinic acid (citrovorum factor), and Horowitz, Doevschuk, and King used labeled glucose to establish the pathway for C biosynthesis, the last step of which (L-gulonolactone to L-ascorbate) was not performed by primates, guinea-pigs, fruit-bats, or the red-vented bilbul bird (8336). However, by 1974, Wilson (8336) had established that female guinea-pigs and women could biosynthesize minimal amounts of C under extreme duress.

Human requirements for C could not be quantified until C had been fully identified. In 1936, van Eekelen estimated 60 mg/day; in 1940, Crandon suggested 30 - 45 mg/day, noting that great depletion was necessary to produce scurvy (8336). Thus, debate started on what should be the optimal tissue level of C, saturation, or the minimum to avoid signs of fatal depletion (scurvy); in 1949, Bourne argued that an intake of 1 - 2 g/day was physiological (1037). The remainder of this section will take up that debate.

B. Special Effects (Foods)

In 1949, Hohl et al. (3635) compared the C contents of frozen fruit and vegetable purees prepared by various methods, and found very wide variations of C content (see original paper for details).

the British Navy replaced its Spanish sweet limes with bitter limes from the West Indies (0591) these were given to the Nares expedition of 1875 and found to be less effective than the Spanish limes, which were given to the Ross expedition (0591) but possibly this was due to adulteration ( ).

In 1883, Barlow described scurvy in infants (1339) which was rife, perhaps because mothers boiled orange juice to sterilize it (8336) [see Breakdown section]

In 1907, Holst and Frolich produced scurvy in guinea pigs (8336).

A disastrous theory then arose that scurvy was caused by a "Bacillus scorbutus", which, in 1916, Jackson and Moore claimed to have identified as a diplococcus; thus Scott took no antiscorbutics on his fatal 1912 journey to the South Pole (8336).

In 1919, McCarrison demonstrated adrenal involvement in scurvy (8336).

In 1920, the Lister Institute, London, attributed scurvy "to a deficiency of a certain accessory factor known as the scurvy vitamine" found in all vegetables and in small amounts in meat and milk; it was sensitive to heat and alkalis (0591), and Bassett-Smith (0591) commented on the interlocking spectrum of vitamin deficiencies (Table 52).

Table 52.

Showing Affinities and Pathologic Features of Scurvy, Beri-beri, etc.(0591)

	Rickets	Infantile Scurvy	Scurvy	Guinea Pig Scurvy	Ship Beri-beri	Beri-beri	Polyneuritis gallinarum
Bone lesions at epiphyses of long bones	+	+	*	+			
Bone lesion at junction of ribs and cartilage	+	+	+	+			
Subperiosteal haemorrhages		+	+	+			
Joint, subserous, subcutaneous and muscle haemorrhages		+	+	+	+		
Spongy gums		+	+	+	+		
Nerve degeneration			+	+	+	+	+
Cardiac hypertrophy and degeneration			+	+	+	+	+
Dropsy				+	+	+	+
Palsy						+	+

*Depending on age of patient

VI. Consumer Exposure

A. History of Vitamin C

Human requirements of Vitamin C are controversial: to correct any impression that high intakes were first advocated by Pauling (6329), a brief chronology has been compiled from several sources (0591,1339,8336, 9024, 9407).

Scurvy signs were described by Hippocrates (ca. 460 - 377 B.C.) (8336).

In 1497, Vasco da Gama sailed around Africa and lost 62% of his crew to scurvy (1339) (8336). In 1535, the Cartier expedition to North America suffered from scurvy and was taught by Indians to decoct Ameda leaves, possibly spruce (8336) or pine (1339).

The observations of Andrew Boord in 1552 did not include scurvy (8336) but by 1564, the Dutch knew that it was countered by fresh vegetables and fruit (0591). In 1593, Hawkins used lemon juice on his voyage to the South Seas (1339) but he thought scurvy was due to salt exposure (8336); Albertus, however, in 1593, described scurvy signs that responded to sour juices (9024). In 1740-44, Anson sailed around the world and noted that scurvy retarded the healing of wounds (8336).

In 1720, Kramer recognized scurvy as a deficiency disorder (9024); in 1734, Bachstrom identified the deficiency as one of fresh vegetables and fruit (8336) and rejected explanations of exposure to salt or cold climate (1339). In 1747, Lind confirmed Bachstrom's conclusions by his experiments with citrus fruit, published in 1753 (8336). Lind recommended preserved citrus juice, and he and Trotter warned against using lead-glazed ware (0591). In 1770, Stark died while making himself experimentally deficient (8336).

Preserved citrus prevented scurvy deaths, though not scurvy, during Cook's voyages of 1773-1774 (0591). Lind's student, Blane, became Commissioner of the British Navy Board and, after a successful 23-week voyage in 1782, added lemon juice to the Navy diet in 1796; lemons and sweet limes were imported from Spain (0591), limes by 1804 (1339).

In 1845-1846, the Irish potato famine was accompanied by scurvy, and potatoes were recognized as antiscorbutic (8336).

In 1854, a British Merchant Shipping Act required the provision of antiscorbutics on any ship away from port for more than 10 days (8336).

In 1860, Hirsch concluded that scurvy appeared when a "special ingredient" was lacking in the diet and disappeared when it was added (1339). In the same year,

Tumor-active Agents

In 1963 Bobb (0908) found that C protected Neurospora crassa cultures against toxicity from a carcinogen, 3'-methyl-4-monomethylaminoazobenzene.

In 1970 Pandya et al. (6262) gave benzanthrone 25 mg i.p. to 400-g with or without 50 mg of C p.o. Benzanthrone decreased the C levels of blood, adrenals, and liver, and the C restored the blood levels more than it did the adrenal or liver levels. Deaths from benzanthrone 500 mg/kg were 40% fewer in control guinea-pigs than in scorbutic animals.

In 1972 Zannoni et al. (9368) depleted guinea-pigs of C for 21 days and found that when liver C reached about 3.5 μg/g wet weight (one-third normal), drug oxidation (aniline, aminopyrene, p-nitroanisole) diminished markedly along with decreases of electron transport components such as cytochromes P-450 and b_5 and the NADPH reductase enzymes, and alterations of microsomal-binding absorbance-spectra. Although restoration of C returned liver C levels to normal in three days, restoration of drug oxidation activities required six days, and the authors also observed that the animals had shown no appreciable weight loss or scurvy symptoms. They commented that subclinical C-deficiency might affect human capacities to metabolize drugs especially during periods of growth.

In 1967 Pipkin et al. (6497) studied the urinary excretion of 3-hydroxyanthranilic acid by patients with bladder tumors. They found that 3-HOA decomposed significantly during 6 hours incubation at 37°C, but that high levels of urinary C produced by pretreatment of the patients inhibited this decomposition.

Ultraviolet Irradiation

In 1939 Jungeblut and Feiner (4050) reported that prolonged UV irradiation of guinea-pigs, rabbits, and rhesus monkeys produced no change of tissue C levels unless the skin was burned, when the C level fell.

In 1963 Chandrasekhara et al. (1424) found that either of two sulfa drugs, sulfasuccidine or sulfaguanidine, depressed the liver and urinary levels of endogenous C in rats. These depressions were counteracted by additional dietary protein. Lactose supplements were able to prevent but not counteract the depression of liver and urinary C, and other vitamins were without effect.

Thyroid Hormone

A.80401 (8612). In 1968 Pokotilenko and Poliachenko gave rats 25 mg/kg C s.c. and/or thyroid 0.15 g/100 g to study oxidative phosphorylation in cerebral mitochondria during thyrotoxicosis. C failed to affect the toxic actions of thyroid but enhanced O_2 absorption and PO_4 esterification slightly.

In 1969 Goldman and Gould (3044) reported that the C requirements of guinea-pigs were increased when they were fed thyroid supplements.

Tobacco

In 1970 Hughes et al. (3757) tested the effect of experimental inhalation of tobacco smoke on guinea-pigs receiving standard intakes of C. Adrenal C levels were depressed after 4 days, and after 18 days the adrenals were hypertrophied as well as being C-depleted.

In 1970 Pelletier (6362) compared, in man, the C status of tobacco smokers and nonsmokers. Lower initial blood and urinary C levels were found in the smokers, but no differences were found after six days saturation with 2.2 g L-ascorbate. C-loading tests indicated that the initial differences were not due to prior differences of C intakes, and further tests after desaturation (judged by excretion) showed that smokers excreted 10% less C than nonsmokers. The author speculated that in smokers, C was either less available or was utilized somewhat differently than in nonsmokers.

In 1970 Bailey et al. (0432) gave controlled exercises to smoking and nonsmoking young male volunteers who also received either 2 g/day C p.o. or a placebo. The C produced no significant differences of respiratory adjustment or O_2 utilization in either group, smokers or nonsmokers.

In 1960 Burns et al. (1266) found that a number of chemically unrelated drugs stimulated L-ascorbate biosynthesis in rats. These included: chloretone, meprobamate, phenylbutazone, chlorcycline, diphenhydramine, orphenadrine, 3-methylcholanthrene, and 3,4-benzpyrene. Barbital and chloretone also enhanced synthesis of free D-glucuronate and L-gulonate; renal factors and drug glucuronide formation were ruled out, and the authors suggested that glucose metabolism might be the source.

In 1961 Conney et al. (1653) found that drugs that induced increases of liver microsome drug-metabolizing enzymes in rats also increased the endogenous synthesis of L-ascorbate from D-glucose and D-galatose via the glucuronic acid pathway. Such drugs included:

1. Barbital and chloretone.
2. Analgesics: aminopyrine and antipyrine.
3. Muscle relaxants: orphenadrine and meprobamate.
4. Antirheumatics: phenylbutazone and oxyphenbutazone.
5. The uricosuric, sulfinpyrazone.
6. Antihistamines: diphenhydramine and chlorcyclizine.
7. Carcinogens: 3-methylcholanthrene and 3,4-benzpyrene.

The authors surmised that the enzyme inductions included the pathway for L-ascorbate synthesis. They reported that in guinea-pigs C-deficiency reduced the liver microsomal enzymes for metabolism of the muscle relaxant zoxazolamine and made the animals more sensitive to this drug.

Sulfa Drugs

In 1944 Karel and Chapman (4161) found that neither the i.p. injection of three or more doses of 10 mg C, nor 10-day depletion of C, altered the LD_{50} of sulfanilamide for fed or fasted guinea-pigs.

In 1945 Lundquist and Phillips (5089) found that succinyl sulfathiazole 2 g/day increased plasma C in newborn calves but even 15 g/day did not do so in 8-9-month heifers. Sulfapyridine at 15 g/day could increase subnormal plasma C levels but not normal levels; sulfathiazole 15 g/day or chlorbutanol 3 g/day increased plasma C, but if sulfathiazole was fed first, chlorbutanol reduced the plasma C level. This did not happen if succinyl sulfathiazole was fed first.

In 1970 Sharaf and Gomaa (7627) found in castrated rats that C was not androgenic by itself, yet it potentiated the androgenic effects of testosterone.

In 1971 Sharaf and Gomaa (7628) found similarly in ovariectomized rats that C was not estrogenic, yet it acted synergistically with estradiol.

In 1950 Nadel et al. (5888) found adrenal C levels of 144±5 mg% in his growing female guinea-pigs. Depletion by diet reduced this 32% in one day and 95% in 33 days, and hypertrophied the adrenals. Stilbestrol increased the adrenal size and lowered the C concentration, whatever the C intake, for it prevented the restoration of C in the adrenals when depleted animals were refed C.

In 1971 Kalesh et al. (4095) found that platelet C levels in women taking oral contraceptives were more readily depleted than in controls, when placed on low-C diet. They argued that this could make the propositae more liable to thromboembolisms.

In 1972 Briggs and Briggs (1117) measured plasma, leucocyte, and platelet C in 89 women of European, African or Asian descent who were pregnant, or receiving steroid contraceptives, or were controls. Significantly the lowest values were found in those taking oral steroid contraceptives. The authors concluded that the steroids increased C breakdown, and that women taking them orally required more dietary C than usual.

Shared Effects

In 1940 Longenecker et al. (5021) studied C excretion by rats, and found it enhanced by:

1. Barbiturates and their derivatives.
2. Hypnotics -- paraldehyde and chloretone.
3. Antipyretics -- especially aminopyrine and antipyrine.
4. Slightly, by phenols, salicylates, sulfanilamide, sulfapyridine, narcotine, nicotinic acid.
5. Not, by hydroyethylapocupreine or various alkaloids. The authors observed no correlation between degrees of nerve depression and enhancement of C excretion, and no evidence that urinary C was conjugated with any of the drugs. Biosynthesis of C by these rats was reportedly not affected.

A.60311 (8612). In 1966 Biswas and Deb reported that C diminished the testicular degeneration and adrenal hypertrophy induced by feeding tyrosine to rats, concluding that the effect of C was indirect, via the pituitary-adrenal system.

A.60298 (8612). In 1966 Esh and Bhattacharya gave C 0.1-4 mg/100 g q.i.d. to growing guinea-pigs, with a diet containing 10-25% of either casein or peanut protein. Protein economy and utilization was improved by C in all experimental groups, by a number of standard criteria. Dose-dependent increases of aspartate and alanine aminotransferases occurred in liver, heart, kidney, and plasma.

Psychotropic Drugs

In 1959 Sapeika (7300) reported that chlorpromazine and iproniazid but not chloroquine diminished adrenal C but not liver C in rats. Three monoamines, adrenaline, noradrenaline, and serotonin, given s.c., also diminished adrenal but not liver C.

In 1968 Rajalakshmi and Patel (6734) found that rat liver biosynthesis of C and its concentration was transiently increased, after a dose of C, by fluphenazine, chlorpromazine or triflupromazine, but only fluphenazine increased the C concentration in brain and adrenals. In brain most increase was found in olfactory lobes and hypothalamus, and least in basal ganglia and visual cortex.

In 1969 Bardhan and Chatterjee (0548) reported that in female rats C, 1 g/kg/day i.m. did not influence the adrenal hypertrophy and ovarian function inhibition produced by reserpine 0.25 mg/kg/day s.c.

Salicylates

H.60144 (8612). In 1966 Schless et al. gave sodium p-aminosalicylate 6-7.5 g 2-4 times daily with/without 6 g C four times daily p.o. to volunteers; blood PAS levels were increased by C, absorption and excretion of PAS were diminished.

Sex Hormones

A.60202 (8612). In 1966 Chatterjee and Bardhan gave rats 100 mg C q.i.d., i.m., together with diethylstilbestrol 0.2 mg alternate days s.c. to study the effects of estrogen-stress on the adrenals. They found that C counteracted some stress effects, and concluded that C decreased the release of ACTH from the pituitary.

(8101) found in guinea pigs that C alone did not reduce nitrite-induced methemoglobinemia but was effective synergistically with methionine.

Other Vitamins

In 1957 Brown et al. (1172) reported that a mixture of tocopherol, citrate and ascorbate was "about 80 times more effective" at retarding oxidation in vitro than was citrate or ascorbate alone.

In 1944 Milhorat (5559) briefly reported an in vitro color reaction (yellow) produced when water was added to a dry mixture of C with nicotinamide or nicotinic acid. He commented that he did not know if this was biologically significant.

In 1951 Welch et al. (9060) found that urinary excretion of the folic acid metabolite, citrovorum factor, was enhanced by administration of C in normal rats and humans, in a dose-dependent manner attributed to a role of C in the metabolism of folic acid. This augmentation was not found in patients with scurvy.

In 1951 Holly (3644) treated patients with megaloblastic anemia in pregnancy with C, B_{12}, or both, and found that the combined therapy was more effective than either vitamin given alone.

In 1973 Bieri (0827) suggested that "the ingestion of large doses of vitamins C or E could appreciably reduce an individual's vitamin A status."

In 1958 Cox et al. (1694) studied interactions between C and B_{12} given to humans. When B_{12} was deficient, C disappeared more rapidly from plasma, and more so when the patient had megaloblastic anemia. C metabolism was "corrected" by B_{12} therapy.

H.90116 (8612). In 1969 Aizicovici et al. claimed that a mixture of other vitamins potentiated blood C increases in man after doses of C.

Phenols

A.70165 (8612). In 1967 Subaschandran and Balloun gave acetyl-p-aminophenol and C to heat-stressed poultry. C decreased the cloacal temperature with or without the acetyl-p-aminophenol; no interaction was reported.

Proteins, Amino Acids, and Amines

I.70055 (8612). In 1967 Lassina induced lipolysis in vitro with catecholamines; this could be reversed by calcium chloride only with additions of C.

Metals - Arsenic

In 1945 McChesney (5383) studied the *in vitro* and *in vivo* (rats) interactions of As and C, using neoarsphenamine. He found that C retarded oxidation of the As compound (particularly as to the *o*-aminophenol groups) and attributed this to decrease of the redox potential of the blood. Animal evidence suggested that the site was the bloodstream, not liver or kidney, and retention of As by these organs was unaffected by the presence of C.

Nitrites

In 1972 Mirvish *et al.* (5619) found that reactions between C and nitrites blocked the formation of N-nitroso compounds *in vitro*, to an extent that depended on the compound and experimental conditions. For these experiments nitrous acid was reacted with oxytetracycline, morpholine, piperazine, *N*-methylaniline, methylurea, or dimethylamine. Less effective blocking was obtained with urea or ammonium sulfamate. The authors commented that C was already added to nitrite-preserved meats for coloring purposes, and that further studies should therefore be practical.

In 1972 Bolyai *et al.* (0967) studied the effects of sodium nitrite and C on methemoglobin production in guinea pig and human red cells *in vitro* and on guinea pigs *in vivo*. *In vitro*, when the nitrite converted one-third of guinea pig blood pigment to methemoglobin, C in concentrations of 0.05 (physiological) to 5.0 mM had no attenuating effect. In human red cells methemoglobin formation was attenuated by C 5.0 mM but not by less. In other experiments C at 5.0 and 0.5 mM reduced already formed methemoglobin in human red cells. *In vivo*, in guinea pigs, no difference in methemoglobin production was found between C-deficient and C-replete states.

In 1970 Kociba and Sleight (4439) found that 45-50 mg/kg of nitrite ($NaNO_2$) was uniformly more toxic to guinea pigs that were C-deficient than to controls, causing higher levels of methemoglobinemia. Methylene blue was protective at 10 mg/kg, as pretreatment. The toxicity was expressed as death rate in one experiment, and reproductive failures in another.

In 1970 Stoewsand (8100) reported that C added as 0.5% of the diet of Japanese quail had no effect on methemoglobinemia or nitrite deposition in eggs when nitrite was also fed as 0.5% of the diet. In a further report, Stoewsand *et al.*

In 1941 Marchmont-Robinson (5230) reported that C 50 mg/day protected workers exposed to lead against the "usual effects" of absorption but did not diminish the level of lead in the urine.

Metals - Fluorine

In 1934 Phillips et al. (6455) using guinea pigs reported parallel effects on cell respiration between fluorine toxicosis and C-deficiency.

Metals - Cadmium

In 1970 Fox and Dry (2585) reported that cadmium fed at toxic levels to young Japanese quail for 4 weeks produced stunting, anemia, and high Cd with low Fe levels in the livers. When C was added to the diet, the blood and growth effects were counteracted, but not the liver findings, and the authors concluded that C was among the requirements for adequate protection against environmental cadmium.

Metals - Vanadium

In 1954 Mitchell and Floyd (5630) found that C was more effective than $CaNa_2EDTA$ at protecting mice against the toxicity of $NaVO_3.H_2O$, and they inferred that C acted to reduce the vanadium.

Metals - Mercury

In 1964 Mokranjac and Petrovic (5665) determined that mercuric chloride 14.3 mg/kg was the minimum LD_{100} for their guinea pigs. Then:

1. Animals given 0.2 g/day C p.o. starting 5 days before the MLD of $HgCl_2$, all survived.
2. When C was given from the time of $HgCl_2$, nine of 25 animals survived.
3. When one dose of 1 g C was given along with $HgCl_2$, two of ten animals survived.
4. When 0.2 g/day C was given for six days before $HgCl_2$ and nil afterwards, 18 of 20 survived.

The authors concluded that C was an antidote to $HgCl_2$ poisoning, by some mechanism other than direct reduction of the salt to an insoluble compound.

Confirmatory reports will be found in the bibliography.

In 1967 Lee et al. (4806) found in fasting nonanemic subjects given 200 mg $FeSO_4$, that absorption of the Fe was enhanced by 1 g of C but not by 50 mg. H.80279 (8612). In 1968 Rodrigues gave anemic patients $FeSO_4$ 131 mg with/without C 500 mg p.o. four times daily and found that C increased the hematinic effect of the $FeSO_4$ by about 50%.
H.80037 (8612). In 1968 McCurdy and Dern found that C potentiated the absorption of $FeSO_4$ by human subjects regardless of the respective times of administration.

In 1969 Smith et al. (7884) found in rats, by everted sac methods, that C enhanced transfer of Fe from mucosal to serosal surface, without evidence for an active transport system.

In 1971 Lipschitz et al. (4968) found that C deficiency in guinea pigs increased their storage of iron in the spleen and diminished it in liver. In both organs ferritin was diminished and hemosiderin increased. Replacement of C restored the normal distribution of C. Other experiments suggested that siderosis in the African Bantu might result partly from C deficiency.

In 1971 Wilson and Loh (9149) gave old people C 500 mg/day and/or Fe 105 mg/day p.o. and measured leucocyte C and hemoglobin levels for 14 weeks. The C raised the leucocyte C; when Fe was added it lowered the C level but in females this recovered. Iron with/without C raised the hemoglobins, and in females C alone did this (but not so much as C with Fe). The stability of the hemopoietic process was altered, and again there were sex differences.

Metals - Lead

In 1940 Pillemer et al. (6489) compared the effects of basic lead carbonate on a total of 88 guinea pigs subclinically depleted of C, with the effects on a total of 48 guinea pigs fed to saturation with C. They found that the high C level did not alter weight loss or blood effects of the Pb but did prevent its effects on nerves. The lead did not affect utilization or metabolism of C, or complement activity of the serum (reported as C-dependent). The authors commented that for therapy of lead poisoning, removal of the cause was more efficient than administration of C.

In 1939 Holmes et al. (3654) found that C 100 mg/day diminished toxic signs in 34 factory workers and 3 painters exposed to lead absorption.

in 100% O_2 at 45 psia, and response curves were constructed. The authors concluded that C and aspirin possibly increased endogenous H_2O_2 synthesis and might be detrimental to persons exposed occupationally to high-O_2 environments.

Hypo-/hyper-glycemic Agents

In 1948 Sherry and Ralli (7660) found that insulin s.c., i.v., or i.m., caused plasma and urinary C levels to fall promptly, but transiently, in man, dog, and rat. In vitro and in vivo studies on these species and guinea-pigs showed no in vitro effects of insulin, and no effect on biosynthesis of C. The authors concluded that insulin caused a transient transfer of C from plasma into the tissues, for the insulin also increased leucocyte-platelet C levels without affecting rbc C.

A.70499 (8612). In 1967 Fabianek et al. gave rats insulin and C or thiourea to study the permeability of dermal connective tissue. Neither drug altered the response to insulin.

A.90177 (8612). In 1969 Fiedler injected guinea-pigs with oxyquinoline, C, or $ZnSO_4$ in a diabetogenic experiment. Oxyquinoline briefly enhanced blood glucose, and C did not affect this response.

Metals - Iron

A.70065 (8612). In 1967 Reeber et al. found in gastrectomized rats that C increased Fe absorption from iron dextran but only to normal values.

I.80042 (8612). In 1968 Hopping and Ruliffson found in rats that a mixture of C and citric acid enhanced the intestinal absorption of $FeCl_3$.

A.80322 (8612). In 1968 Forth et al. gave female rats $FeCl_3$ and reported that C, nicotinic acid, citric acid, or EDTA decreased retention of the Fe. The compounds were given p.o., and the authors commented that they did not observe the site of retention in the small bowel.

In 1966 Fujino et al. (2661) found correlations between estrogen activity, plasma C, plasma Fe, hematocrit, and hemoglobin in six normally menstruating young women. Plasma Fe rose and fell with plasma C although total Fe-binding capacity altered inversely with plasma Fe and C.

A.70482 (8612). In 1967 Manolova studied the immunization of rabbits against diphtheria. A mixture of vitamins including C increased the production of antitoxin in rabbits primed with antitoxin and O-antigens.

Histamine

In 1973 Zuskin et al. (9457) studied the interactions of histamine and C on airway constriction in 17 healthy humans and on contractions of guinea pig tracheal strips in vitro. They found that C 0.5 g, p.o., reduced histamine-induced constrictions in man, and that propanolol 80 mg p.o. did not block this effect. In vitro, C relaxed the tissue, and blocked the histamine-induced contractions, but the effect was less in the presence of 2.5 µg of propanolol.

Hydrocarbons

In 1971 Jenkins et al. (3970) exposed guinea-pigs of both sexes to vapor of mineral spirits at 900 mg/m^3 for 90 days, and found that animals fed diets fortified with higher levels of C (in the range 0.00015-2.0% of diet) were less susceptible to toxicity of the vapor. Males were more susceptible than females, and the NMRI:(ASH) strain of guinea-pig more than the FTD:Hartley strain. The authors stated that their findings emphasized the importance of dietary Cx for species that depended on exogenous sources of C.

In 1970 van Heyningen (8714) found that cataracts tended to develop in the lenses of rabbits fed naphthalene 1 g/kg/day, but if not, the C content increased progressively. From in vitro studies the author concluded that C was oxidized in the system by naphthalene metabolites, entered the lens as dehydroascorbate, and was there reduced to C by glutathione.

Hydrogen Peroxide

In 1969 Miller (5577) found that a mixture of C and H_2O_2 was effective against gram-negative bacteria, rendering them sensitive to lysis by lysozyme. As the effect was not enhanced by horseradish peroxidase, the authors inferred that the mixture probably generated short-lived free radicals that attacked the cell wall.

In 1971 Serrill et al. (7575) injected aspirin and C into normal and E-deficient mice and found that the drug combination increased rbc lysis by H_2O_2 in vitro. Both treatment and E-deficiency diminished the survival of such mice

Dimethysulfoxide

In 1967 Archer et al. (0284) examined wound biopsies in rats given topical 90% dimethylsulfoxide (DMSO) with or without C i.p. before biopsy in an experiment designed with additional control groups. They concluded that DMSO dilated the endoplasmic reticulum and diminished the ribosomes, as reported in scorbutic guinea pigs without DMSO. These effects were partly prevented by administration of C.

Diphenylhydantoin

In 1973 Stambaugh et al. (8028) reported a case in which gingival hyperplasia attributed to Dilantin therapy was associated with concomitant depletion of C in the tissues, responding to C p.o. 1 g/day.

Drugs Active in Shock and Immunology

I.70057 (8612). In 1967 Pittilo and Lucas found that bacteria could be sensitized to radiation by 1-β-D-arabinofuranosylcytosine. Sensitization was inhibited by cysteine or C.

A.60329 (8612). In 1966 Dawson et al. found that pyrilamine alone did not affect bradykinin anaphylaxis in rats but potentiated its counteraction by C. They concluded that the effect was to reduce the level of bradykinin.

In 1965 Dawson and West reported that C with mepyramine protected guinea pigs from anaphylactic shock, and C alone protected them from anaphylactic reactions to dextran. However, C did not protect them against convulsions produced by histamine aerosols. Dietary C level influenced the rate of decarboxylation of histidine but not the activity of histaminase.

A.60320 (8612). In 1966 Vodokhlebova found that a mixture of compounds including C increased the signs of traumatic shock in rabbits.

A.90082 (8612). In 1969 Laborit et al. gave anesthetized rabbits one or more of epinephrine 10 μg, tyrosine 2 mg/kg, and C 50 mg, all i.v. to study neural and cardiovascular effects in hemorrhagic shock. C potentiated the responses to tyrosine, but neither tyrosine nor C affected the responses to epinephrine.

H.90087 (8612). In 1969 Laborit and Baron gave rabbits various combinations of eight compounds including C to study catecholamine movements after hemorrhagic shock. Tyramine added to a combination of tyrosine and C elicited paroxysms and EEG changes.

In 1953 Harris et al. (3423) also found no evidence of interaction in guinea pigs, but reported massive liver enlargement after ACTH, which they did not find in rats. They speculated that the liver enlargement in the guinea pigs was edematous.

In 1971 Ginter et al. (2904) reported that guinea pigs with experimental atheromata induced by cholesterol retained more C in their tissues than controls.

In 1954 McSwiney et al. (5345) found in human patients and guinea pigs that when tissues were adequately saturated with C, corticotrophins briefly augmented the urinary excretion of reduced C and its immediate metabolites; when not, no effect.

In 1952 Bacchus et al. (0385) reported that in female rats C diminished the urinary excretion of 17-ketosteroid metabolites of injected cortisone during the 24 hours after treatment.

In 1952 Bacchus et al. (0382) found that glycogen deposition in the livers of adrenalectomized mice was enhanced when C was added to cortisone treatments.

In 1953 Lovell et al. (5035) found in rheumatoid arthritis patients that C status, depletion or saturation, had no effect on the condition or on the therapeutic effects of ACTH and cortisone. Other reports to the same effect were found.

In 1953 Stewart et al. (8089) reported that ACTH given to man enhanced the plasma levels of C while decreasing the dehydro moiety of the C.

In 1955 Haynes and Scheid (3484) did not find the effect described by Bacchus et al. above when massive doses of C were given to human patients receiving hydrocortisone therapy.

In 1952 Booker et al. (0994) reported that cortisone therapy increased the level and duration of blood cell and plasma responses to C in man, and that while ascorbic acid alone did not alter serum cholesterol levels, C given to patients on cortisone therapy elevated the serum cholesterol.

Dieldrin

In 1971 Wagstaff and Street (8949) studied in guinea pigs the interaction between dieldrin and C on induction of liver microsomal hydroxylating enzyme systems. C deficiency impaired the induction by dieldrin in 2 days; maintenance of induction was related to dietary levels of C and not to the liver concentrations of C. Higher intakes were required to maintain induction than to prevent scurvy.

Convulsant and Anticonvulsant Drugs

In 1943 Emmett et al. (2326) performed feeding experiments with Na-diphenylhydantoinate, or dilantin sodium, on the utilization of C by young guinea-pigs. The animals were deprived of C, or were fed only 0.5 mg/day, or were fed 50 or 100 mg/kg/day, for 7-9 weeks. In another series, somewhat different treatments were continued up to 14 weeks. The authors reported that the dilantin sodium had no influence on C levels in plasma, adrenals, brain, testes, or liver, and thus probably had no influence on utilization of C by the guinea-pig.

In 1965 Dey (2010) incubated samples of strychnine with lemon juice (containing C and acylase enzyme) and bioassayed the mixture by s.c. injection into mice of amounts thought to supply 2 mg/kg strychnine. Samples incubated at 37°C had almost lost their convulsive and lethal properties, but those incubated at 50°C had retained those properties. Samples incubated with plasma from cat, rat, pigeon, or toad, also retained those properties. Animals given C 1-2 g/kg were protected. The author concluded that strychnine was metabolized mainly by deacetylation, and that C possibly protected against strychnine binding in vivo.

A.60169 (8612). In 1966 Grosman exposed mice to turpentine and strychnine and treated them with hydrocortisone, adding one or another of certain vitamins. Added C increased the mortality; only added riboflavin reduced it.

A.60199 (8612). In 1966 Dey gave rats 2 MLD of tetanus toxoid and found that C 1g/kg i.p. or i.v. twice daily counteracted lethality; he reported no toxic signs but mild local tetany, and stated that C could also be used prophylactically.

In 1967 Dey (2012) reported that C (50-2000 mg/kg) or its precursors L-gulonolactone or 2-ketogulonolactone (1-2 g/kg) prevented or countered the toxic effects of strychnine in mice. The protection was abolished by ethionine, and C was ineffective against other convulsants such as Picrotoxin or Metrazol. Conclusion: that C probably metabolized strychnine to some non-toxic derivative.

Corticotrophins and Sterols

In 1953 Schilling et al. (7391) reported no interactions between C and ACTH in a wound-healing study on guinea-pigs.

Carbon Tetracholoride and Ethionine

In 1969 Soliman et al. (7924) found that C 30 mg/mouse protected against experimental liver cell damage by CCl_4 8 ml/kg more effectively than nicotinic acid 50 mg/mouse or E 100 mg/mouse, but less effectively than total belladonna alkaloids 0.05 mg/mouse.

In 1961 Bernheim (0736) found that CCl_4 depressed the C content of rat livers, but ethionine did not. Chloretone increased the C content but not after it had been depressed by CCl_4, and not after ethionine (when chloretone depressed the C content). The author commented that the "apparent reversal" of the action of chloretone on C by CCl_4 or ethionine was unexplained.

In 1967 Chatterjee and Bardhan (1451) reported that C 1 g/kg b.d., i.m., protected rats against CCl_4 3 ml/kg i.p.

In 1967 Kato (4205) reported that in rats:

1. Ethionine decreased liver C and increased urinary C.
2. CCl_4 decreased both liver and kidney C and urine C.
3. Ethionine + C prevented increases of urinary C and induction of drug-metabolizing enzymes by chloretone, barbiturate, or methylcholanthrene.
4. CCl_4 + C only partly prevented these increases.

The author concluded that ethionine interfered with storage of C, while CCl_4 damaged the liver so as to diminish synthesis of C.

In 1968 Nachtomi et al. (5887) reported that rat and chick livers responded differently to ethylenedibromide and CCl_4 toxicities. CCl_4 in rat liver depressed C levels more than did EDB, while chick liver C was less affected by either poison. Both poisons increased APase activity in rat liver and decreased it in chick liver.

A.90158 (8612). In 1969 Leber et al. gave fasted scorbutic guinea-pigs one or more of C, dehydro-C, and ethionine, i.p., to study effects on liver microsomal mixed-function oxidases. C increased the hydroxylation of acetanilide and cytochrome P-450 activity, counteracted by ethionine. Dehydro-C increased acetanilide hydroxylation and aminopurine; ethionine did not affect this, but C then increased P-450 and mixed-function oxidase activities.

In 1957 Franz et al. (2606) reported a pilot clinical study in which 89 volunteers received a placebo, a bioflavonoid, C, or C plus bioflavonoid for three months, and reported the onset and identity of various common-cold symptoms. The bioflavonoid was described as an extract of grapefruit peel, called Narangin, but was not further identified, and its daily dose was 333 mg. The daily dose of C was 65 mg. The authors reported that the C treatment did not affect the incidence of colds but accelerated recovery, and that the bioflavonoid had no effect alone or with C, either on the cold symptoms or on the blood ascorbate level.

In 1966 Hudspeth et al. (3734) compared C with a citrus bioflavonoid hesperidin for effects on capillary fragility in broiler poultry as measured by rate of healing of bruises. Each compound was added to feed at various levels, and the authors concluded that neither was beneficial.

Blood Coagulation-active Drugs

In 1970 Owen et al. (6216) added ascorbate in vitro to normal blood from rats, dogs, and humans in order to prevent destruction of serotonin. They reported that 2 mg C eliminated the anticoagulant effect of heparin 0.01 mg.

In 1971 Rosenthal (7063) reported the case of a 52-year-old woman whose prothrombin time, on warfarin therapy, was decreased from 23 seconds to 12 seconds when she started taking non-prescribed supplements of C. When the supplements were stopped, the prothrombin time recovered rapidly to 28 seconds.

In 1972 Smith et al. (7872) reported a case of a 70-year-old woman with thrombophlebitis in which very high intake of C, 16 g/day, apparently prevented an increase in prothrombin time from warfarin medication until the dose was stepped up to 25 mg/day. On cessation of C supplements warfarin could be maintained at 10 mg/day. However, when rabbits were given up to 250 mg/kg of C their response to warfarin up to 0.8 mg/kg orally or i.v. was unaffected. The authors urged further clinical studies of warfarin in patients with high intakes of C.

Table 51. Ascorbic Acid Concentration of Whole Blood, of Plasma, and of Urine (9234)

Date 1941	A				B				C			
	Whole blood mg%	Plasma mg%	Urine mg%	Urine 24 hr mg	Whole blood mg%	Plasma mg%	Urine mg%	Urine 24 hr mg	Whole blood mg%	Plasma mg%	Urine mg%	Urine 24 hr mg
2-5	.84	.40	.61	5.20	.63	.22	.38	4.40				
2-6	1.22	.46	.45	5.48	1.12	.31	.32	4.13				
2-7	1.27	.43	.46	4.80	.86	.33	.28	3.47				
2-8	.99	.43	.39	4.84	1.20	.40	.34	2.43				
2-9	1.12	.58	.35	3.94	.89	.55	.20	1.86	1.12	.58	.34	2.90
2-10	.96	.40	.69	7.54	1.07	.34	.58	6.84	1.20	.54	.89	6.30
2-11	1.37	.42	.48	6.73	.63	.39	.31	3.91	1.37	.60	.66	2.15
2-12	1.36	.40	.48	4.90	1.04	.37	.27	4.90	1.25	.55	.43	3.30
2-13	.79	.34	.46	2.59	.71	.36	.44	5.20	1.22	.50	.45	4.50
2-14	.76	.30	.43	4.18	.73	.30	.31	3.85	.92	.51	.40	3.89
2-15	.77	.32	.50	5.76	.64	.21	.35	3.89	1.00	.47	.43	4.65
Phenobarbital gr iii h.s.												
2-16	.56	.32	.19	2.31	.62	.23	.12	2.18	.68	.52	.28	3.43
2-17	.86	.26	.25	3.53	.76	.14	.27	2.50	.95	.39	.47	3.60
2-18	.80	.31	.49	6.32	.66	.19	.42	5.75	.80	.38	.68	5.66
2-19	.63	.16	.41	5.27	.57	.14	.28	5.34	.70	.32	.53	5.68
2-20	.73	.25	.31	4.30	.52	.16	.27	3.70	.56	.35	.56	4.92
Drug discontinued												
2-21	1.10	.24	.24	2.64	.72	.23	.22	2.92	1.03	.33	.38	3.40
2-22	.60	.20	.32	5.51	.65	.20	.25	3.40	.73	.38	.54	3.80
2-23	.64	.27	.25	3.47	.53	.23	.20	5.30	.74	.32	.36	3.97
2-24	.67	.25	.35	3.50	.67	.15	.34	3.59	.81	.32	.52	3.30
2-25	1.05	.20	.33	4.95	1.11	.15	.24	2.40				
2-26	.50	.16	.24	3.66	.46	.16	.24	2.40				
2-27	.64	.16	.13	1.15	.64	.13	.24	2.40				
Avg												
1st control	1.04	.41	.48	5.09	.87	.34	.34	4.08	1.15	.54	.52	3.96
Drug	.72	.26	.33	4.35	.63	.17	.27	3.89	.74	.39	.50	4.65
2nd control	.74	.21	.27	4.55	.68	.18	.25	3.20	.83	.34	.45	3.62

Bioflavonoids

A.60242 (8612). In 1966 Robbins found in guinea-pigs that hesperidin reduced capillary resistance and cell aggregation, C also reduced cell aggregation and capillary fragility, and hesperidin and two other flavonoids (rutin and naringin) synergized with C.

A.70458 (8612). In 1967 Berezovskaya reported that catechins (bioflavonoids) significantly depressed the oxidation of thyroxine by C in liver.

A.70282 (8612). In 1967 Berezovskaya reported that C potentiated the inhibition of hyaluronidase by bioflavonoids but was itself non-inhibitory.

A.70347 (8612). In 1967 Blane and VanderMuel gave heat-stressed guinea-pigs C and/or trihydroxyethylrutoside (a flavonoid) and reported that heat caused C to move from adrenals to liver; the flavonoid synergistically enhanced liver C content.

H.00027 (8612). In 1970 Almaula and Shah gave chloramphenicol with/without a mixture of C and amphotericin to volunteers; the mixture lowered the peak and prolonged the duration of blood chloramphenicol concentrations.

Aspirin

In 1971 Serrill et al. (7575) reported that acetylsalicylic acid and C, in mice, accelerated hemolysic responses to hyperbaric oxygen (100% O_2) mediated by H_2O_2. The authors cautioned that this could imply danger to humans exposed to hyperoxia.

Barbiturates

In 1972 Wade et al. (8938) reported that C-deficiency in guinea pigs depressed hydroxylation of aniline and barbiturate drugs in liver. Neither specific enzyme activities nor that of cytochrome P-450 were affected, but the amount of microsomal protein was diminished by C deficiency.

In 1941 Green and Musulin (3172) reported that the higher the C level in guinea pigs, the less the depression produced by barbiturates. However, barbiturates did not cause C depletion when given frequently. The authors inferred that these were metabolic effects rather than direct chemical interactions between C and the barbiturates.

In 1941 Wright et al. (9234) fed three volunteers the diet in Table 50 described as C-free diet for 10 days (control period), then added phenobartital 180 mg/day for 5 days, then repeated the control period. From Table 51 the authors concluded that phenobarbital did not affect the whole blood, plasma, or urinary C level, and that supplementation of the control diet with crystalline C 25 mg/day was insufficient to maintain C levels in whole blood or plasma.

Table 50. C-Free Diet (9234)

Food	g	Food	g	Food	g
Am. cheese	20	Figs, dried	30	Pork	75
Bacon	10	Flour	3	Puffed wheat	15
Beef	75	Grape jelly	30	Ripe olives	30
Butter	45	Grape juice	100	Soda crackers	12
Cream	170	Jello	16	Sugar	13
Dates	20	Limas, dried	20	Walnuts	15
Eggs	2	Macaroni	10	W. W. bread	120
Evap. milk	200	Peas, dried	20	Total calories	3021

Aniline Poisoning

In 1962 Magos and Szizia (5140) reported that C reduced the amount of methemoglobinemia resulting from aniline in rats, but not the duration of this response; Heinz body formation was "intensified", and the authors cautioned against the use of C as therapy for chemical syanosis.

Antibiotics

In 1963 Boyd (1047) reported that clinical and pathological signs of the toxicity of benzylpenicillin, in oral doses of 0.1 of the L-LD_{50} given to rats, were not affected by the C content of the diet, and therefore the drug did not affect the capacity of these rats to synthesize C.

In 1966 Polin et al. (6549) reported that C at 100 mg/kg of diet partly counteracted the adverse effects of thiabendazole (an antibiotic and vermicide) fed at 0.2-0.4% of the diet to chicks. These effects included depressed hemoglobin and hematocrit (not counteracted by C, but by B_{12} or K) and internal hemorrhaging (counteracted by C): when the three vitamins were given together, the birds had no ill effects. Without these vitamins, hematopathy was seen at 0.05-0.1% thiabendazole.

In 1971 Polec et al. (6541) prevented azotemia and other evidence of nephrotoxicity induced in rats and dogs by pure tetracycline-HCl i.v. 50 mg/kg, with concomitant administration of C at 125 mg/kg or more. D-isoascorbate gave similar protection to rats, and mannitol to dogs but not rats. As C was ineffective when renal blood flow was restricted, but otherwise increased the dialysis rate of H^3-tetracycline, the protection was inferred to be secondary to an osmotic diuretic effect.

H.70325 (8612). In 1967 Modr et al. gave normal subjects test doses of potassium phenoxymethylpenicillin p.o. and found that its immediate rise in the blood was enhanced by C.

H.70362 (8612) In 1967 Borisova gave patients syntomycin or levomycin for therapy and reported that additions of thiamine and C diminished the side effects.

H.80201 (8612). In 1968 Shar et al. gave human volunteers C and/or tetracycline-HCl 1 g/day p.o. in four doses and reported that tetracycline potentiated plasma and buffy-layer increases of C concentration.

H.80237 (8612). In 1968 Vedmina et al. claimed that a vitamin mixture including C raised the blood antibiotic level after administration of an antibiotic mixture.

V. Drug Interactions

A number of reports have been abstracted from the compendium, Drug Interactions (8612); these are prefixed by a letter and reference number. The letter is A (animal study), or H (human study), or I (in vitro study).

Alcohol

In 1953 Forbes and Duncan (2570) gave rats and guinea pigs, i.p., aqueous solutions of approximately 50% ethanol after overnight fast, and measured adrenal cholesterol and ascorbate levels 24 hours later. Both sexes were used. In other experiments 3 g EtOH were diluted 1:1 in 5% glucose or 0.85% saline. Reductions of 36-40% in adrenal ascorbate were attributed to the alcohol, not the dilutant. The authors previously observed similar reductions after oral administration of similar solutions.

In 1961 Czaja and Kalant (1752) reported that ethanol, 2 g/kg, decreased adrenal C and cholesterol levels in rats when given i.p. but not when given by tube. Gavage affected the levels more than did the alcohol. Prior anesthetization eliminated pain as a cause of these findings, and the authors were able to confirm that they were due to different rates of absorption of the EtOH into the blood.

In 1969 Tamura et al. (8305) tested C alone, and C plus a glucose-cysteine mixture, against the acute toxicity of acetaldehyde and nicotine solutions injected into mice. They reported that the C-glucose-cysteine combination reduced the death-rate after acetaldehyde and increased the survival time after nicotine.

In 1960 Lester et al. (4864) found that 85 alcoholic patients excreted less C than 23 control subjects and more patients than subjects were C-deficient. Saturation values were obtained in both groups by giving C 500 mg/day for a week (but not less) before placing them on a maintenance regimen, suggested as 150 mg/day.

H.80010 (8612). In 1968 Camps and Robinson gave 12 volunteers ethanol and either C or fructose orally and i.v. and concluded there were no interactions.

In 1968 Pawan (6336) found that a mixture of vitamins including C at 200 or 600 mg/day had no effect on the rate of metabolism of ethanol in man.

In 1972 Evets and Korablev (2396) reported that C counteracted side effects of N,N,N',N'-tetraethylthiuram disulfide, used in therapy of alcoholism. Anerobic glycolysis and transketolase activity were inhibited in rats.

Table 49 Some Enzymes Influenced by Ascorbic Acid

Enzyme & Tissue	Animal	Level of C	Conclusions	Year	Reference
Heart-muscle Lipoprotein lipase	Baboons	0; 34 mg/kg/day	C increased serum C, decreased LPL activity.	1973	4513
Muscle creatine-phosphokinase	Guinea pigs	ca. 0.05; 0.5 mg/ guinea-pig/day	C required for optimal enzyme activity	1970	2058
Brain & liver Na-K-ATPase & Mg-ATPase	Rabbit	10^{-4} M in vitro	Inhibited Na-K-ATPase and (less) Mg-ATPase	1970	3830
Olfactory & brain Na-K ATPase & Mg^{++} ATPase	Rabbit	0; 4; 20 (x 10^{-5} M) in vitro	Partial inhibition of Na-K ATPase; stimulation of Mg^{++} ATPase.	1972	4436
Testicular Δ^5-3β-hydroxysteroid dehydrogenase	Toad	200 µg/100 mg of slices in vitro	Stimulation by dehydro-ascorbate, not by ascor-bate.	1970	0871
Liver p-hydroxy-phenylpyruvic acid oxidase	Guinea pig	25 mg/animal/day; scorbutic controls	Protects from inhibitions, so maintaining normal metabolism of tyrosine.	1960	9367
Plasma alkaline & acid phosphatases, succinic dehydrog-enase	Guinea pig	Scorbutic; fed 5 mg/day/animal; inj. 1 mg/g BW for 5 days	All activities maintained by C.	1968	0868
Brain, liver, adrenal tryptophan & tyrosine hydrox-ylases	Guinea pig	Normal vs.deficient	More activity in normals	1970	5923
Hexose diphosphatase	In vitro	10^{-6} to 10^{-3} M	Possibly competitive with substrate.	1948	8978

Table 48. Effects of Ascorbic Acid on Some Other Vitamins

Vitamin	Animal	Level of C	Findings and Conclusions	Year	Reference
Thiamin	Rat	1% or 5% in diet	C had a thiamin-sparing effect, but not when injected.	1974	5851
Pantothenic acid	Rat	2% of a 65% sucrose diet (pantothenic acid deficient)	Delayed or suppressed signs of pantothenic acid deficiency.	1957	0544
B_{12}	Man	"Massive"	Scurvy cured, but serum C level remained low until B_{12} stores were replenished.	1966	4074
B_{12}	Man	75-200 mg/day	Pernicious anemia; plasma C levels remained low until B_{12} deficiency was abolished.	1968	4075
Folic acid	Man	1 g/day	When pteroxylglutamic acid was given, excretion of citrovorum factor remained low until C status was replete. Conclusion: that C provides for conversion of PGA to CF.	1952	2701
E	Lamb	Low, in diet	Plasma C but not other tissue levels of C were enhanced by E, thought to influence C biosynthesis "loosely".	1972	1890
K	In vitro	0.02 M	C and K together were cofactors for ATP-dependent reduction of NAD by coenzyme Q reductase.	1964	7265

Table 47. Some Effects of Ascorbic Acid Upon the Blood ( )

Blood Component	Animal	Effect and Conclusions	Year	Reference
Platelets	Guinea pig	C deficiency increased platelet concentration and prothrombin time; neither C replacement nor K supplements corrected terminal signs.	1959	0561
	Man	Scurvy reduced platelet adhesiveness, rapidly corrected by C. Conclusion: possible factor in bleeding defect of scurvy; experimental scurvy in guinea pigs may differ from human scurvy.	1967	9152
Hemoglobin	Man	In patients with methemoglobinemia C, rapidly lowered MHb concentration and related symptoms.	1946	1342
	Man	MHb of 81 healthy neonates was 0.75% of total Hb; in distressed neonates it was 0.95%, and C 50 mg/day p.o. had no effect.	1968	6477
	Guinea pig & Man	When MHb concentration in C-deficient guinea pigs was enhanced by sodium nitrite, C had no effect *in vivo* or *in vitro*. High concentrations of C (5 mM) did reduce nitrite-induced MHb in human rbc *in vitro*, but the chemistry was judged to be complex.	1972	0967
Vessels	Guinea pig	C-deficient guinea pigs had focal lesions of the capillaries; losses of basement membrane and pericapillary collagen were seen by electron microscope.	1968	3102
	Man	Though C is needed for metabolism of dopamine to norepinephrine *in vitro*, C deficiency did not lower endogenous norepinephrine but did decrease blood-vessel response to the hormone.	1970	0008

Table 46. Some Effects of C on Hormonal Systems

System	Animal	Effect and Conclusions	Year	Reference
Thyroid	Rat	Adrenalectomized, given cortisone or DCA; thyroidectomized, given thyroid and/or C. Conclusion: corticoids assist cold-resistance through "conditioning"; C assists same through thyroid hormones.	1956	1981
Adrenals	Rat	Adrenal C and cholesterol were depleted by stress, unless rats were pre-treated with C.	1969	6537
	Guinea pig	Conclusion: C not involved in corticosteroid synthesis; C deficiency increases adrenocortical activity so that output exceeds possible rate of synthesis.	1970	3621
	Rat & cockerel	Testosterone (5 mg) or corticosterone (0.05 mg) increased the dehydro-C ratio in lymphoid organs. Conclusion: this ratio is important during growth and function of lymphoids.	1971	2060
	Man	C 4g/day p.o. for 4 days increased urinary 11-oxysteroids and decreased 17-ketosteroids, attributed to direct action on adrenal cortex.	1952	4223
Thymus	Guinea pig	Thymic C decreased, and the dehydro-C: C ratio increased according to C intake. Conclusion: C was involved in production of thymic humoral factor, but not in expression of its activity in lymphoid tissues (additional data).	1971	2059

Table 45. Effects of Ascorbic Acid on Some Infections Other Than the Common Cold

Infection	Animal	Level of C	Findings and Conclusions	Year	Reference
Tetanus toxin	Rat	1 g/kg i.p.	Counteracted neurotoxic effects of 2 MLD of the toxin.	1966	2011
Distemper	Dog	1-2.5 g/day i.v. for 3 days or more	Recovery rate of dogs that convulsed rose from 14% to 60%.	1969	4878
Diphtheria toxin	Guinea pig	10-20 mg/animal	Increased animal's resistance without altering toxin or antitoxins.	1935	3179
Malaria parasites	Monkeys	80-100 mg i.m.	Equivocal	1946	5423
Tubercle bacilli	Guinea pigs	As in diet	Less C found in organs of infected animals, indicating increased utilization.	1967	7616
	Guinea pigs	Supplemented diets	C "usually" protected animals from intestinal ulcers when tubercle bacilli were fed.	1933	5390
Influenza A virus	Mice	0.05 M *in vitro*	Inactivated 10 MLD of virus; inferred due to H_2O_2 formed by oxidation of C.	1945	4389

Table 45. Some Effects of C on the Metabolism of Some Major Nutrients

Nutrient	Animal	Findings and Conclusions	Year	Reference
Carbohydrate	In vitro	Autoanalysis for serum glucose was not affected by high serum C levels.	1973	4210
	Man	Ketolytic action; oxidation of acetoacetate except at high concentrations of C (>0.002<0.003 M).	1952	7680
	Man	500 mg x 4/day to 21 obese Ss. Normalized the insulin-glucose tolerance curve but not the glucose tolerance curve.	1956	0287
	Man	C was synergistic with insulin when both were given together.	1952	0598
	Man	No effect on urinary glucose from 1.5 g of C.	1966	2984
	Rat, rabbit	C increased liver glycogen in normals and alloxan-diabetics, independently of insulin.	1958	0704
Amino acids	Chick tibia in vitro	C stimulated hydroxylation of peptide-bound proline, so stimulating collagen synthesis.	1966	3964
		Other bone proteins, DNA, and RNA were also affected.		3963
	Guinea pig liver in vitro	Coenzyme role in tyrosine metabolism.	1952	7511
	Guinea pigs	Protein metabolism and liver enzyme activities.	1966	2369
Fatty acids	In vitro	Long chain fatty acids were oxidized to CO_2 in the presence of C and oxygen.	1957	2822

Table 44. Some Effects of C on Various Disorders

Condition	Animal	Findings and Conclusions	Year	Reference
Hypercholesterol-emia	Guinea pig	In chronic C deficiency catabolism of cholesterol to bile acids was decreased. A requirement of C for cholesterol hydroxylation was inferred.	1971	2897
	Man	C 300 mg/day for 47 days decreased serum cholesterol during seasonal C deficiency with high serum cholesterol, in a group aged over 40 with large intakes of sucrose and animal fats.	1970	2894
	Man	Plasma C levels in a surveyed (not treated) group were not associated with plasma cholesterol levels, but plasma C levels decreased according to increasing cigarette consumption.	1970	2318
Psychopathies	Rat	C 500 mg/kg i.p. increased brain C levels and norepinephrine levels in brain tissues; dopamine levels were decreased.	1968	3907
	Man	40 patients were found to have "subscurvy" and elevated "demand" for C; saturation with 1 g/day improved depressive, manic, and paranoid symptomatologies.	1963	5587
	Man	8 normals required 4 g of C/day (divided doses) to achieve a C excretion threshold; 10 schizophrenics required 40 g/day (average). Therefore they metabolized 10-fold more C than the normals. Clinical improvement was "selective".	1966	8730
Hyperpigmentation of skin	Guinea pig	C, 5-100 times the antiscorbutic dose did not influence oestrogenic pigmentation.	1934	3923
	Man	C found to be associated with melanin in skin, especially in Addison's disease.	1937	1675
	Man & Guinea pig	C was found to have a bleaching effect; the 3-phosphate salt was found to be stable in cosmetics and to be dephosphorylated by cell phosphatases.	1971	8291

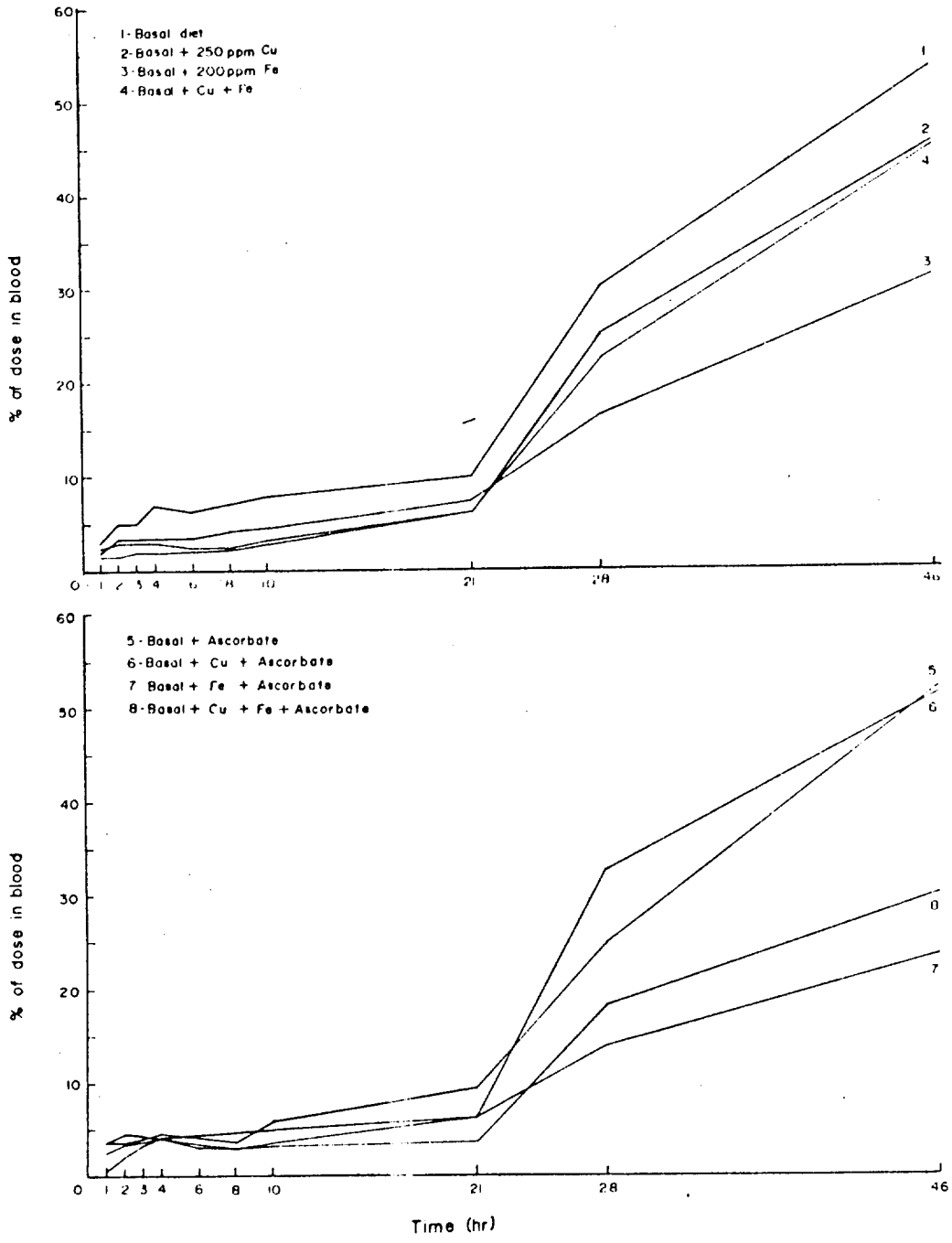

Figure 17. The Appearance of an Oral Dose of ^{61}Cu in the Blood (% of dose) (2905)

In 1974 Gipp et al. (2905) studied the effects of high dietary C on Fe and Cu metabolism in young pigs. High Cu (250 ppm in diet) induced manifestations of Fe deficiency without blocking Fe release from the reticuloendothelial system. High C (0.5% in diet) increased transport and utilization of Fe without altering its level in liver or intestine. The authors concluded that high Cu impaired Fe absorption, and that high C counteracted this impairment. The trends are shown in Figures 16 and 17.

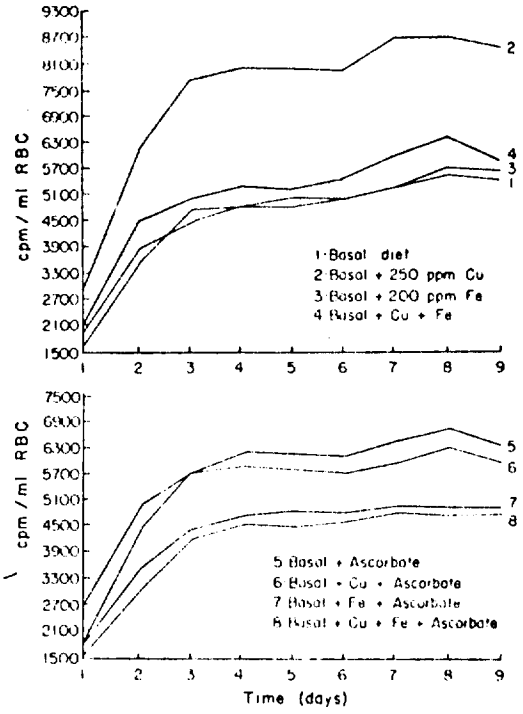

Figure 16. Red Cell Utilization of ^{59}Fe (2905)

Other areas of reported involvement of C are summarized in Tables 44-49.

3. In 1964 Hornbuckle et al. (3683) showed that after experimental wounds of bone or soft tissue, body stores of C were released into the plasma.
4. In 1967 Chvapil et al. (1572), using rats, found that the amount of collagen in the lungs during experimental silicosis was related to the amount of C but not to amounts of other organic acids, and that C influenced collagen formation only in tissues with relatively high contents of collagen. These findings were confirmed in guinea pigs by Kaw and Zaidi in 1969 (4213), who additionally noted an increased retention of C by the experimental group.
5. In 1968 Richmond and Stokstad (6921) showed that the formation of skin collagens and acid mucopolysaccharides was drastically reduced by C deficiency.

In 1970 ten patients aged 5-27 with osteogenesis imperfecta were given C 1 g/day by Winterfeldt et al. (9163), and after three months they were still retaining 225-612 mg/day; the fracture rate was dramatically less than before C therapy, and the authors postulated a collagen effect.

In 1970 Parfitt and Speirs (6277) reviewed the causes of chronic periodontal disease and concluded that no case had been made out for nutritional deficiencies as general initiators in North America. They attributed the generality of cases to local irritants and failures to clean the teeth; "prevention or treatment by means of nutritional supplements like vitamin C is therefore unwarranted unless a specific deficiency of the nutrient can be demonstrated."

However, in 1969 Chaudhry (1471) had found an average C content of 0.37 mg% in the plasma of 102 patients with pyorrhea alveolaris, compared with 0.85 mg% in controls, and noted that the low average was not influenced by age or sex.

Information continues to become available about the various roles of C in metabolic systems. A recent example follows.

C deficiency was often found in situations involving tissue repair, and was considered more frequent in certain segments of thepopulation -- probably in the elderly, possibly in males, and possibly in smokers as opposed to non-smokers. More speculative, according to the author, were claims for a role in prevention of cardiovascular disease and for improvement of mental performance. However, evidence for usefulness in the common cold, rheumatoid arthritis, and bone growth was considered promising, although still inconclusive.

In 1974 Wilson (9146) summarized the involvements of C as follows:

1. Ovarian function
2. Hemopoiesis
3. Brain metabolism
4. Corticosteroid release
5. Liver metabolism

The types of involvement included reducing activity, cholesterol synthesis, fatty acid metabolism, lipoprotein synthesis, metabolism of plasma and tissue proteins, and, via insulin, carbohydrate metabolism. The formation and growth of epithelial basement membranes was C-dependent, especially in the respiratory tract. Although C was not specifically viricidal to common cold viruses, it inhibited many microorganisms, e.g., it prevented replication of RNA and DNA bacteriophage viruses, and there was preliminary evidence that some immunological mechanisms were enhanced by C.

Clinically, doses of C that produced undesirable side effects were those that abnormally elevated plasma C levels. The author concluded that certain of the signs of the common cold were similar to, indeed indistinguishable from, signs of scurvy.

Research into the identity and actions of C grew out of recognition that scurvy was a major sign of a deficiency.. The other major sign to be recognized in early times was delayed wound healing (see brief chronology in Consumer Exposure section). Laboratory studies on wound healing in guinea pigs have included:

1. In 1945 Danielli et al. (1801) showed that slower healing of skin wounds was accompanied by lower activity of phosphatase enzymes.
2. In 1960 Abt et al. (0056) showed that in areas of scar formation the highest concentration of C was in the connective tissue.

6. In 1967, Takenouchi et al. (8297) found that about 80% of large doses of C given to patients was excreted in urine as dehydroascorbic acid. Oral doses were 3 g, and other doses were injected by various routes into human and animal subjects. In man, 3 g/day of C resulted in no increase in urinary oxalic acid (baseline excretion was 20 mg/day), but 9 g of C p.o. or i.m. resulted in urinary oxalic acid 30 mg/day. When a lead ball was placed in the bladders of animals, rats or guinea pigs, i.v. injection of 0.5 mg C/day produced calculus seven-fold the weight of that in control animals, and containing 0.73 mg of oxalic acid versus 0.33 mg.

7. In 1971, Lamden (4702) commented that massive intakes of C "by anyone not under the close scrutiny of a physician should be emphatically discouraged."

8. In 1974, Wilson (9146) stated that excretion of C varied from 1-3% of minimal doses to 60-80% of large doses. The metabolites excreted in urine included dehydroascorbic acid, 2,3-diketogulonic acid, and oxalic acid. About 40% of urinary oxalate came form C. Thus, according to the author, unjustifiedly large intakes of C could result in oxalate stones. C itself was excreted when body stores exceeded 1.5 g and plasma levels exceeded about 0.2 mg/100 ml. (See also Biological Data II, 5 and 6.)

IV. Effects on Enzymes and Other Biochemical Parameters

In 1972 Sims (7760) summarized the effects of C on body systems as follows:

1. Reducing activity.
2. Tissue regeneration, collagen formation, bone deposition.
3. Roles in converting folic to folinic acid, and in metabolism of aromatic amino acids.
4. Other roles influencing blood lipid and cholesterol levels, tricarboxylate and Krebs cycles, and thus metabolism of carbohydrates, fats, proteins, minerals, and other vitamins.
5. Absorption and transport of iron.
6. Synthesis of steroids, activity of leucocytes, function of the reticuloendothelium, and formation of antibodies.

2. In 1969, Sasmal et al. (7316) studied the sequence of enzyme-mediated steps in metabolism of C by the rat. Details can be found in the original paper; the enzymes reported on included L-gulonolactone oxidase (EC 1.1.1.38), ascorbate oxidase (EC 1. 10.3.3), uronolactonase (EC 3.1.1.19), D-glucuronolactone reductase (EC 1.1.1.20), L-gulonate dehydrogenase (EC 1.1.1.45), and 3-oxo-L-gulonate dehydrogenase (EC 4.1.1.34).

3. In 1970, Sneer et al. (7897) found that alloxan given to rats increased the blood C levels slowly, the liver C levels rapidly, and the adrenal C levels slowly. Other changes noted in the adrenals are described in the original paper.

4. In 1971, Bhatia et al. (0816) found that C levels in various tissues, but not in adrenals, were increased by dieldrin fed at 30 mg/kg of BW.

B. Excretion

1. In 1939, Guggenheim (3250) reported from Palestine that urinary excretion of C by a sample of the population was greatest during the citrus season and was less in children than adults.

2. In 1947, Klosterman et al. (4412) found that the renal threshold for C excretion in 12 normal adults ranged from 1.0 to 1.3 mg/100 ml of plasma; they inferred that this remained stable over many years.

3. In 1951, Sigurjonsson (7744) loaded five male students with test doses of C, 10 mg/kg, and measured the urinary output of C over time. About 78-88% was excreted within 7 hours, and after full saturation of the tissues, no more than 50-60% was recovered in the urine. The authors concluded that the remainder was either destroyed or incompletely absorbed.

4. In 1971, Loh and Wilson (5005) gave 5 ovulating and 5 nonovulating females 500 mg/day of C through the cycle and concluded that C excretion patterns were hormone-related: around ovulation, C and LH excretion patterns were "closely" similar.

A number of reports emphasize that oxalate is excreted in increasing amounts after massive intakes of C.

5. In 1954, Lamden and Chrystowski (4705) found that 51 healthy adult males daily excreted an average 38.3±1.7 mg of oxalic acid in the urine, without extra-dietary C. Significant increases of urinary oxalic acid were produced by supplements of 4 g or more of C, but not by less.

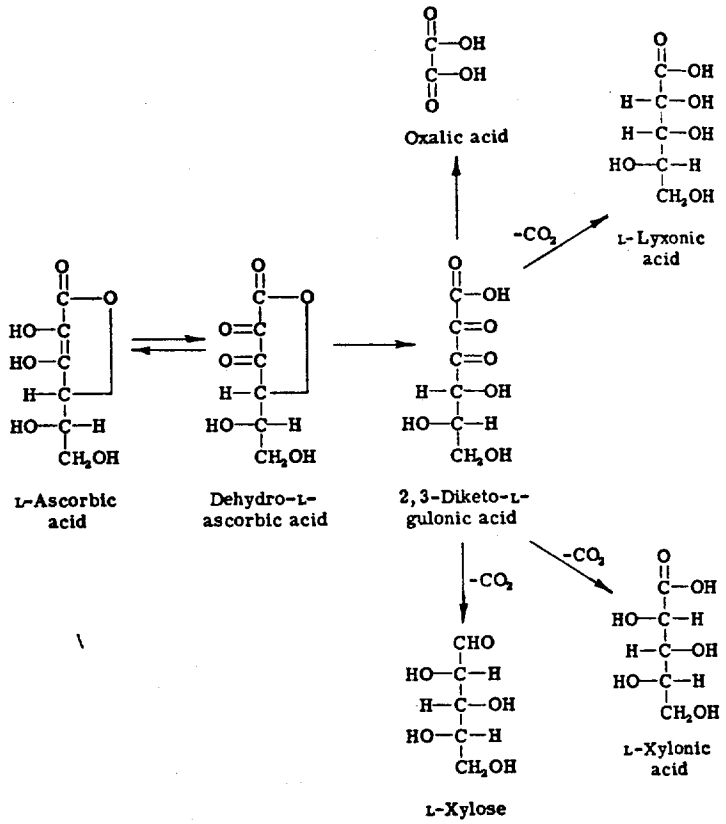

Figure 14. Metabolism of L-Ascorbic Acid (1253)

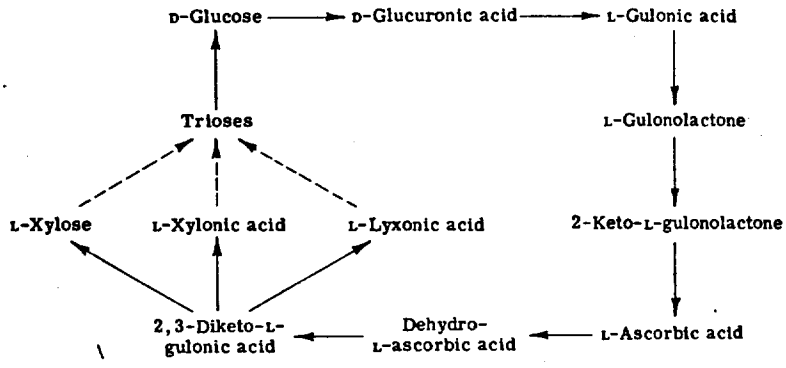

Figure 15. L-Ascorbic Acid as an Intermediate in a Cyclic Pathway of Glucose Metabolism in Animals (1253)

III. Metabolism and Excretion

A. Metabolism

1. In 1967, Burns (1253) reviewed knowledge of the metabolism of C. Its biosynthesis in animals was most likely to follow the pathway shown in Figure 13; in plants the pathway was different and as yet unsettled. Primates and guinea pigs were considered unable to perform the conversion of L-gulonolactone to L-ascorbic acid (more recent findings noted in this monograph have modified this opinion). The metabolism of L-ascorbic acid in animals was considered most likely to follow the pathways in Figure 14, but also to contribute to a cyclic pathway for glucose synthesis as shown in Figure 15. The author also noted that D-ascorbic acid was both oxidized to CO_2 and excreted via the kidneys more rapidly than L-ascorbic acid.

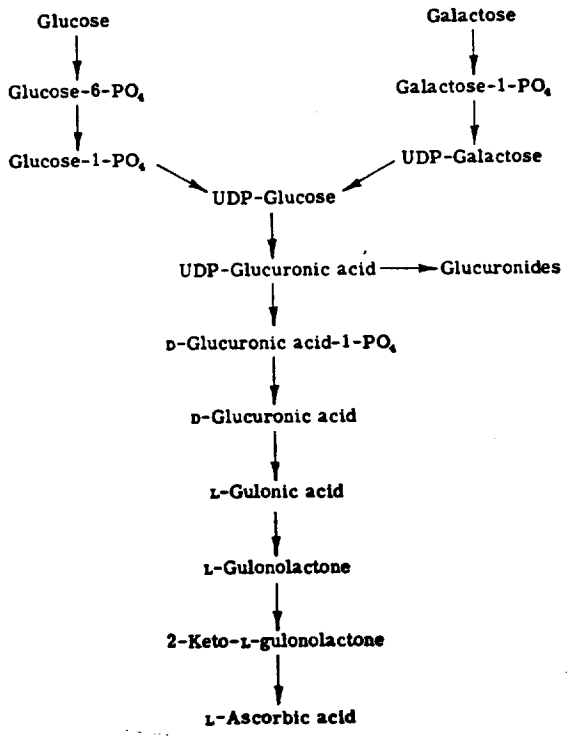

Figure 13. Pathway of C Metabolism (1253)

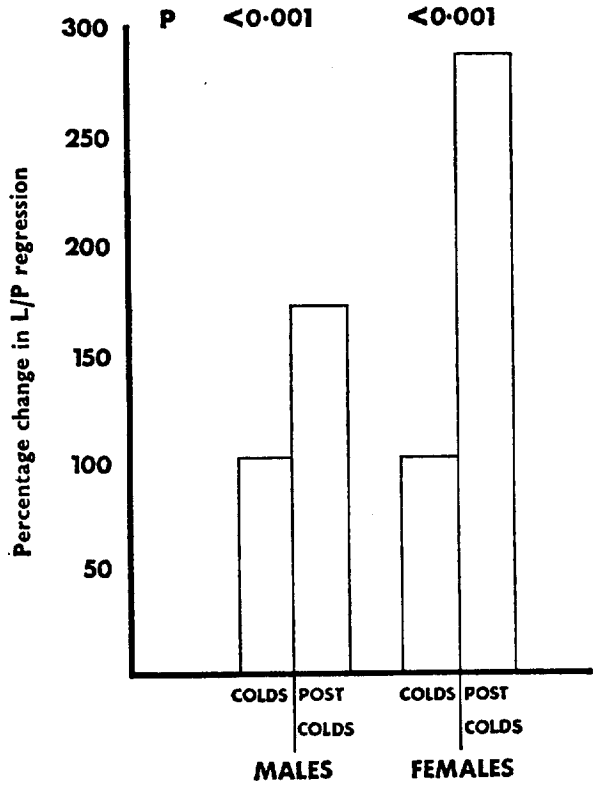

Figure 12. Analysis of the pooled regression coefficients relating leucocyte and plasma ascorbic acid values (L/P regressions) during the ascorbic acid blood-response curves after a loading dose of 500 mg of ascorbic acid in male and female subjects during and following recovery from the common cold. The L/P regressions provide a measure of the transfer of ascorbic acid from the plasma into the leucocytes following the loading dose. (9146).

Table 43. Leucocyte ascorbic acid concentrations. Values related to age in healthy control subjects, in smokers, and related to petechiae and purpura in the elderly. Values four hours after a loading dose of 500 mg of vitamin C during and following common cold, and mean value during and 10 days after onset of cold symptoms. Reported values in gastrointestinal disorders. (9146)

State of health	Leucocyte concentrations (Mean and standard deviations)		Reference
	Male	Female	
Healthy controls (age in years)			
4 to 12	56·4 ± 21·9	—	Loh and Wilson, 1971
11 to 18	36·6 ± 9·1	42·8 ± 12·6	
22 to 49	30·4 ± 8·7	34·0 ± 10·9	
56 to 87	24·4 ± 13·6	23·4 ± 11·3	
Smoking			
Non-smokers	24·6 ± 1·3	30·7 ± 1·4	Brook and Grimshaw, 1968
Moderate	19·6 ± 1·7	25·6 ± 1·6	
Heavy	17·4 ± 3·5	27·8 ± 1·4	
Petechiae and purpura in aged			
Sublingual petechiae (range and mean)	5·1 – 21·3	10·23	Andrews and Brook, 1966
Senile purpura	6·4 – 31·7	16·0	
Positive Hess test	15·2 ± 1·2	-27·9 ± 2·3	Eddy, 1972
Common cold			
During cold—3rd day	24·7 ± 9·7	27·6 ± 5·8	Wilson and Loh, 1974
3 weeks post-cold	32·9 ± 8·6	34·1 ± 11·6	
During cold—1st day	10·3 ± 0·3		Hume and Weyers, 1973
10 days after onset	24·0 ± 6·5		
Peptic ulceration			
Controls	17·9 ± 0·9		Russell *et al.*, 1968
Gastrointestinal haemorrhage	14·2 ± 0·9		
Aspirin or alcohol ingestion	12·6 ± 0·9		
Without history	18·2 ± 0·8		
Duodenal ulceration			
Controls	28·7 ± 8·1		Dymock *et al.*, 1968
Stenosis	11·7 ± 4·5		
Gastric surgery:			
without symptoms	10·1 ± 4·5		Cohen and Duncan, 1967
with symptoms	20·4 ± 8·4		
Duodenal ulcer:			
before surgery	8·7 ± 3·1		
after surgery	12·8 ± 4·3		
Controls	22·9 ± 5·9		
Duodenal ulcer general	10·6 ± 4·9		Esposito and Valentine, 1968
Following gastric surgery	12·7 ± 6·8		Williamson *et al.*, 1967
Controls	22·1 ± 6·4		Esposito and Valentine, 1968
Gastrointestinal disease			
Gastroduodenal disorders	11·0 ± 4·4		Cohen and Duncan, 1967
Intestinal malabsorption	10·7 ± 5·2		Williamson *et al.*, 1967
Controls	20·8 ± 10·5		

Table 26. Clinical Response of Volunteers Who Received Ascorbic Acid Tablets and Were Then Inoculated with Viruses Capable of Causing Colds (8966)

	Results in Volunteers Receiving							
	Rhinoviruses		Influenza B Virus		B814 Virus		All Viruses	
	Ascorbic Acid	Placebo	Ascorbic Acid	Placebo	Ascorbic Acid	Placebo	Ascorbic Acid	Placebo
Number inoculated	29	26	8	8	10	10	47	44
Number of colds	9	9	4	4	5	5	18	18
Mean incubation period (days)	3·1	2·4	3	2·8	3	3·8	3	2·9
Clinical severity of colds: Mild	7	7	1	1	3	3	11	11
Moderate	—	1	—	1	2	—	2	2
Severe	2	1	3	2	—	2	5	5
Mean duration of colds (days)	7·4	9·2	10·7	8·5	6·6	4·6	8	8
Mean score*	6·5	5·9	13·7	9·2	6·2	7·0	8	7
Paper handkerchiefs used daily at peak of cold: Range	4–32	3–56	6–82	10–23	8–25	7–38	4–82	3–56
Mean	10	14	35	17·5	14	19·8	16·5	16·5

* The score is calculated by allotting points for both the number of days on which individual symptoms and signs were recorded and the severity of each.

Table 27. Results of Laboratory Tests on Volunteers Inoculated with Three Different Viruses (8966)

	Results in Volunteers Given									
	Ascorbic Acid					Placebo				
	Total Volunteers	Colds	Virus Isolated	Antibody Rise	Laboratory Evidence of Infection	Total Volunteers	Colds	Virus Isolated	Antibody Rise	Laboratory Evidence of Infection
M Rhinovirus PK	15	5	5	6	9	16	3	5	2/14	5
H " DC	6	2	4	5	6	3	2	2	3	3
Total rhinoviruses	21	7	9	11	15	19	5	7	5/17	8
Influenza B	8	4	5	2	5	8	4	6	1	6

Table 28. Some Previous Controlled Studies on the Effect of Ascorbic Acid on Length of Respiratory Infections (8966)

Population Studied	Type of Illness	Dosage of Ascorbic Acid	Effect Observed
20 volunteers on scorbutogenic diet*	Colds	Continuous 10 or 70 mg. daily	Colds in deprived group lasted 6·4 days (G.M.), in non-deprived 3·3 days. Not statistically significant
Adolescents in institution with low vitamin C intake†	Tonsillitis (probably streptococcal) and colds	Continuous 200→50 mg. daily	No effect on incidence. Tonsillitis shorter in treated groups. Colds unaffected
Members of ski camp‡	Pharyngitis (colds apparently uncommon)	Continuous 1,000 mg. daily	Incidence reduced

* Bartley *et al.* (1953). † Glazebrook and Thomson (1942). ‡ Ritzel (1961).

more so in males than females (Figure 12). Thus more C was needed to maintain tissue saturation. During attacks of asthma C excretion diminished, and recovered during remissions.

Table 42. Means and SD for Hematological and Biochemical Parameters for Masai (n = 21) and Bantu (n = 24) Dietary Groups (1839)

Parameter	Masai Mean	Masai SD	Bantu Mean	Bantu SD	Significance[a]
Age, years	33.9	13.5	32.6	13.5	NS
Hemoglobin, g/100 ml	14.8	1.6	15.1	1.8	NS
RBC, $\times 10^6$/mm^3	4.92	0.54	5.08	0.51	NS
MCV, μ^3	87	4	88	7	NS
PCV, %	42.8	4.5	44.5	4.6	NS
MCHC, %	34.4	0.8	33.9	1.4	NS
MCH, pg	30.0	1.8	29.9	3.1	NS
Platelets, $\times 10^3$/mm^3	222	56	236	60	NS
Leukocytes, /mm^3	7,072	2,479	7,101	2,219	NS
Plasma ascorbate, mg/100 ml	0.16	0.08	0.57	0.49	++
WBC ascorbate, μg/10^8 WBC	13.7	5.8	29.0	12.9	++
Serum iron, μg/100 ml	111	43	123	62	NS
Serum folate, ng/ml	5.5	2.2	6.1	2.7	NS
RBC folate, ng/ml	297	80	404	138	+
Serum B_{12}, pg/ml	1,188	943	607	322	+
Serum protein (total), g/100 ml	8.4	0.8	7.9	0.6	+

[a] The final column gives statistical significance levels of differences between the means of the two groups (NS = not significant; + = $P < 0.02$; ++ = $P < 0.001$)

10. In 1974, Wilson and Greene (9150) studied leucocyte and plasma levels of C after a 2 g loading dose in men and women. They reported that normal women achieved higher leucocyte C levels than did normal men, and that absorption ceased after 2 hours in men and 4 hours in women. During colds a 2 g loading dose increased the C content of leucocytes in women but not in men. However, peak leucocyte levels after continued supplementation with C did not differ between the sexes. Thus the sex difference was a metabolic characteristic. Citing various findings, the authors concluded that humans, as well as guinea pigs, possibly had the capacity to synthesize C and to use it more economically in response to stress or deficiency.

Table 41. Total Ascorbic Acid Concentration in Leucocytes and Organs of Guinea Pigs* (4234)

	Ascorbic acid dose, mg/100 g body weight				
	0.2	0.5	1.0	1.6	10.0
Leukocytes, $\mu g/10^8$ cells	3.83 ± 1.38^a	6.40 ± 1.55^{ab}	8.48 ± 2.86^{bc}	10.10 ± 4.92^{cd}	12.04 ± 3.24^d
Adrenals, mg/100 g	10.65 ± 1.67^a	22.42 ± 6.22^b	30.65 ± 8.70^{bc}	36.60 ± 8.73^c	64.11 ± 16.95^d
Spleen, mg/100 g	5.30 ± 1.58^a	12.00 ± 2.47^b	15.15 ± 4.56^{bc}	16.90 ± 3.29^c	24.52 ± 5.38^d
Brain, mg/100 g	3.49 ± 0.61^a	7.76 ± 1.74^b	9.80 ± 1.66^c	11.92 ± 1.19^d	17.06 ± 1.52^e
Heart, mg/100 g	0.48 ± 0.19^a	1.26 ± 0.21^b	1.56 ± 0.57^{bc}	1.71 ± 0.46^c	2.90 ± 0.48^d

* Each mean ± SD is based on eight observations except for leukocyte TAA concentration at a dose of 0.2 mg/100 g body weight, n = 7; and at 10.0 mg/100 g body weight, n = 6. Means with the same superscript are not significantly different ($P > 0.05$) at each site of measurement.

7. In 1974, Burr et al. (1267) surveyed the plasma and leucocyte C levels in 830 elderly inhabitants of a town in South Wales, and also the consumption of fresh fruit and green vegetables. For detailed findings see original paper. The authors concluded that the C values were lower than those reported in younger persons, and the values declined significantly with age within the range of 65 years and up; also, they were correlated with fruit and vegetable intakes. However, the higher C levels in women than in men, and in nonsmokers versus smokers, were not considered to be fully explained.

8. In 1974, Davies and Newson (1839) compared hematological measurements of Masai tribesmen using a traditional diet high in animal protein, with those of nonpastoral Bantu using a mixed diet, near Nairobi, Kenya. The Masai were judged to consume "well below" the recommended intake of 30 mg/day of C, but showed no signs of scurvy or anemia (Table 42). Their blood picture of low ascorbate and high B_{12} and protein in an apparently healthy people was not clearly understood (1839).

9. In 1974, Wilson (9146) reviewed the distribution of C, noting that both plasma and leucocyte levels of C varied by age and sex (Table 43). Females tended to retain more C than males, especially under desaturating conditions. During viral colds, for example, C was transferred via the plasma to the affected tissues especially to the inflamed tissues during the catarrhal phase,

4. In 1973, Odumosu and Wilson (6107) showed that C-deficient female guinea pigs survived longer than males owing to a small endogenous recovery of tissue C after its initial fall. Some individuals of both sexes were able to utilize small amounts of exogenous gulonolactone under these conditions (Table 40).

Table 40. (1) Mean weights and alterations from mean (g) at days 12 (male) and 20 (female), (2) numbers losing weight and (3) nos remaining alive in groups of six male and female guinea pigs fed on scorbutogenic diets and receiving daily supplements of saline (SS) or gulonolactone (GS) (6107)

	Males						Females					
	SS			GS			SS			GS		
Time on diet (d)	1	2	3	1	2	3	1	2	3	1	2	3
0	484·8±11·5	0	6	467·0±15·6	0	6	460·8±21·7	0	6	459·5±11·5	0	6
6	+29·2	0	6	+25·8	0	6	+7·2	0	6	+3·0	0	6
12	+31·4	3	6	+31·7	2	6	+9·7	1	6	+14·0	0	6
18	−5·6	6	3	−12·8	5	4	+11·0	2	6	+24·0	1	6
20	−64·0	3	3	−13·7	2	4	−7·3	5	6	+37·0	1	6
24	−107·0	3	3	−15·0	2	4	−13·5	4	6	−9·8	3	6
30	—	−	0	−39·5	1	2	−62·6	4	6	−21·3	4	6
36	—	−	0	−68·5	2	2	−12·0	2	2	−12·0	3	5
42	—	−	0	−108·0	2	2	−72·0	1	1	+93·0	3	3
52	—	−	0	+63·0	2	1	−25·0	1	1	+87·0	1	3
60	—	−	0	+35·0	1	0	—	−	0	+87·0	2	3
80	—	−	0	—	−	0	—	−	0	+39·0	3	2
84	—	−	0	—	−	0	—	−	0	+80·0	0	1
90	—	−	0	—	−	0	—	−	0	+85·0	0	1

5. In 1974, Wilson (9146) stated that plasma and leucocyte C levels normally peaked at about 60 $\mu g/10^8$ cells; excesses then appeared in the urine, because plasma levels seldom exceeded the renal threshold.

6. In 1974, Keith and Pelletier (4234) compared the concentrations of C in adrenals, brain, spleen, and heart of growing male guinea pigs with the concentrations in their leucocytes after administration of L-ascorbic acid daily for 42 days at doses from 0.2 to 10.0 mg/100 g. The results (Table 41): that the leucocyte concentrations reflected the tissue concentrations.

2. In 1969, Loh and Wilson (5000) reported that in 23 female and 28 male students there was a circadian fluctuation of taste threshold for C, inversely proportional to plasma C levels.

3. In 1971, Loh and Wilson (5002) concluded that leucocyte C levels did not reliably indicate tissue C status in normal people, but rather the availability of C for tissue storage. When they compared the plasma C and leucocyte C values in supplemented (500 mg/day) versus unsupplemented groups, the leucocyte C range was less in the supplemented groups, indicating leucocyte saturation with C (Figure 11). The plasma and leucocyte values were related in all groups (students, workers, old people with and without supplements) but the relationship differed, and the plasma C values indicated turnover rates under these conditions. The authors commented that the leucocyte C pool was labile, that C was mobile between tissues, and that at least two different parameters were needed to assess the C status of the normal individual.

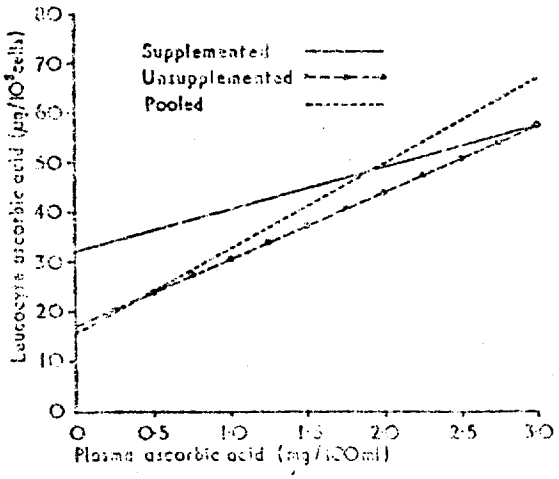

Figure 11. Calculated Regression Lines Relating Leucocyte with Plasma Ascorbic Acid Values in the Group Receiving No Supplementary Vitamin C, in the Group Receiving Supplementary Vitamin C, and in Both Groups Pooled Together (5002)

output from the adrenals as adrenal and plasma C diminished; leucocyte C levels fell more slowly throughout. In a third experiment (6102) they reported that female guinea pigs given a L-gulonolactone oxidase inhibitor lost weight and died with liver C 20% of normal and plasma C zero; however, in a survival test on socrbutogenic diet some individual females recovered plasma and liver C to some extent after the initial fall, and the authors concluded that these had been "able to readjust their ascorbic acid metabolism" in some way to compensate for lack of dietary C.

3. In 1971, Loh and Wilson (5001) found that D- and L-isomers of C were absorbed at similar rates from the human mouth. About 50% was absorbed after 5 minutes retention at the physiological pH 6; at pH 3.4, about 75% was absorbed at 5 minutes, but little more between then and 9 minutes (Table 39).

Table 39. Effect of Contact Time and pH of Ascorbic Acid (AA) Solution on Disappearance of AA from the Mouth and Its Uptake by Buccal Mucosal Cells (BMC) in Human Subjects (mean values and standard deviations) (5001)

		Males		Females	
pH	Contact time (min)	From mouth (μg)	BMC uptake (μg/g)	From mouth (μg)	BMC uptake (μg/g)
3·4	1	110·33±11·46	2·71±0·17	74·80±9·32	1·43±0·14
	2	132·38±13·04	3·73±0·18	112·70±11·71	2·92±0·31
	3	368·25±29·84	4·77±0·17	309·57±24·42	3·65±0·41
	4	572·42±29·40	0·24±0·31	507·27±15·01	4·95±0·60
	5	816·62±12·15	9·74±0·26	706·58±18·38	8·06±0·36
	9	883·57±14·97	10·56±0·40	799·80±17·58	9·09±0·47
3·4	5	826·6±43·2	11·5±0·9	727·6±32·8	8·8±0·5
6·0	5	492·2±24·4	7·0±0·4	443·6±18·7	6·4±0·3

B. Distribution

1. In 1948, Samuels (7263) gave male rats for 5 weeks diets high in protein, fat, or carbohydrate; tissue levels of endogenous C were low with protein diets and in tissues where most amino acids were metabolized. Exogenous C up to 50 mg/day increased C concentrations except in plasma. Conclusion: that C utilization was greatest where most amino acids were metabolized.

Table 38. Effect of Canning and Pasteurization on Vitamin C Content of Foods

Food	Processing Method	Effect	Reference
Grapefruit & orange juice	Canning	10 to 30% loss in vitamin C content	0753
Orange juice	Pasteurization	<1% loss in reduced vitamin C	4557
Grapefruit juice	Canning, commercial	3% loss in ascorbic acid	8945
Grapefruit juice	Pasteurization	No loss in reduced vitamin C	4557
Tomato juice	Canning, home or commercial	Apparently little or no loss; "Good antiscorbutic substances"	0753
Apricots, peaches, plums, cherries	Canning	"Practically free from vitamin C"	0753
Peas, Lima beans, spinach, asparagras	Canning,	50-85% loss in vitamin C	0753
Milk, pasteurized	Pasteurization, commercial	50% loss in ascorbic acid	9176
Milk, pasteurized	Short time-high temp; absence of copper	Only slight inactivation of ascorbic acid	0753
Evaporated milk	Sterilization	22% loss of added vitamin C	1221

II. Absorption and Distribution

A. Absorption

1. In 1938, Abt and Farmer (0046) emphasized that absorption of oral C and plasma C levels were variable in man, and the physiology was unknown. On these grounds they could not accept quantitative statements about tissue saturation based on urinary excretion data until more should be known about degradation of C and its fecal elimination.

2. In 1970, Odumosu and Wilson (6104) found in an intestinal loop experiment in guinea pigs that C absorption was reduced in the terminal stages of scurvy. In another experiment (6103) they found large increases of corticosteroid

Table 37. Vitamin C Losses in Cooking Foods (2317) (Cont'd)

Food Class	Cooking Method	Ascorbic Acid Loss, % Dissolved	Thermal	Total
Potatoes (Cont'd)	Fried			35 27-51
	All methods	? 5*	? 19*	30 0-73
Vegetables other than leafy green or yellow (beets, cauliflower, celery, corn, cucumber, eggplant, lima beans, onions, parsnips, rhubarb, rutabagas, sauerkraut, turnips	Boiled	25 0-41	10 0-17	35 0-77
	Steamed	10** 0-19	10 4-14	30 14-69
	Pressure	5 0-11	15 0-36	35 8-70
	Waterless cooker			55 49&66*
	Sautéed			50 22-60
	Baked			20 12-27**
	All methods	25 0-41	10 0-36	35 0-77
Fruits, other than citrus Apples, all methods of preparation				? 80
Other fruits (i.e., plums, bananas, apricots)				? 30-40
Berries				? 15*
Dried fruits				? 0-5*

* One laboratory
** Inadequate data
See original article for other details.

Table 37. Vitamin C Losses in Cooking Foods (2317)

Food Class	Cooking Method	Ascorbic Acid Loss, % Dissolved	Thermal	Total
Vegetables-leafy green or yellow. Leafy green (beet greens, Brussels sprouts, cabbage, chard, spinach, turnip greens)	Boiled	25 12-66	15 0-56	45 15-78
	Steamed	20 5-50	15 6-26	30 6-76
	Pressure	15* 8&18	5* 2&5	30* 13-53
	Sauteed			55** 49&59
	All methods	25 5-66	15 0-56	45 6-78
Green and yellow (asparagras, green beans, broccoli, kohlrabi, okra, peas, peppers, squash, sweet potatoes	Boiled	25 12-48	10 2-45	40 6-69
	Steamed	10 0&18*	15 14&14*	25 14&32
	Sautéed			50 0-76
	Baked			40 6-72**
	Pressure			40** 12-58
	Waterless cooker			50** 48-50
	All methods	25 0-48	10 2-45	40 0-76
Potatoes	Boiled	? .5*	? 19*	25 0-46
	Baked			35 0-73

Drying, as indicated above, results in almost complete destruction of vitamin C in foods due to oxidation (4337). Although orange juice and certain acid fruits have been desiccated experimentally with complete or moderate reduction of vitamin C activity (0753,3385), no information was found in the literature review that commercial procedures effective in this respect have been developed (0753).

Gamma-irradiation of table-ripe tomatoes with doses of 50, 200, 300, and 400 Krad caused reduction in ascorbic acid of 6, 21, 12, and 24%, respectively, in a recent (1968) study (0009). The authors reported that their results agreed with those for peaches, cherries, oranges, strawberries, and mangos published by other workers; references are given in original article (0009).

Cooking destroys varying amounts of vitamin C as indicated by King and Tressler above (4337). Ascorbic acid is readily oxidized and loss during heating is now known to be an oxidative process (6184). The main oxidation product under acidic conditions is dehydroascorbic acid; in alkaline solution, hydrogen peroxide, oxalate ion, and L-threonate ion are formed (6184).

Vitamin C tablets. Compressed tablets composed of approximately 25 mg of ascorbic acid and an equal amount of lactose (excepient) stored in a dish exposed to air over a period of 6 months lost practically no ascorbic acid according to the 2,6-dichlorophenol indophenol titration method (7556).

In view of the foregoing, the overages necessary to meet label claims for the required shelf life of many foods vary with the nature of the product (2690).

Table 36. Vitamin C Content of Various Foods After Storage or Processing

Food	Findings and Conclusions	Year	Reference
Orange- & grapefruit juice canned commercially	1 g/day protected guinea pigs for 90 days, when slightly over 1 g/day of canned slices was needed.	1932	2460
Tomatoes, home canned; tomato juice canned commercially	No significant loss during canning at home; 30-50% loss after shelf storage at room temperature for six months. Commercially canned tomato juice, opened, no loss after 4 days in refrigerator; but juice from commercially canned tomatoes had significant loss after 2 days in refrigerator.	1939	5402
Cabbage	Losses in cooking up to 50%, in baking up to 80%.	1946	8835
Citrus fruits in processing	Acid juices retained ca.96.5%, less acid ca.92.9%. Less retained with sodium benzoate than with SO_2. Deaeration and addition of sugar improved retention. Flash pasteurization retained more than slow pasteurization (of bottles).	1952	6672
Orange juice frozen commercially	Stored for 5 years at -4°, 5°, and 10° F. "Ascorbic acid was found to be very stable".	1957	4273

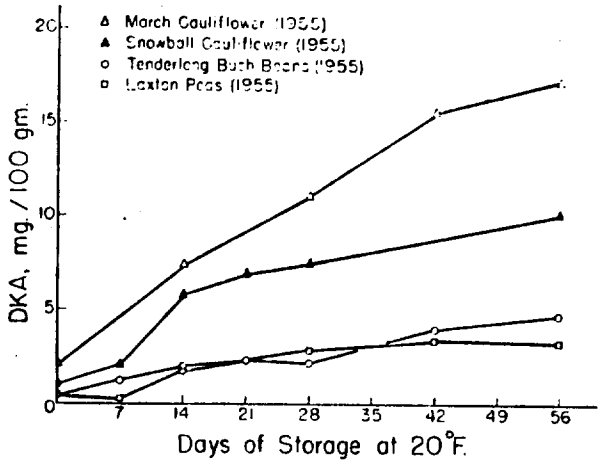

Figure 10. Effect of Storage at 20° F on the 2,3-diketogulonic Acid (DKA) Content of Cauliflower, Bush Beans, and Peas (2063)

In 1965 Grant and Alburn (3158) found that nonenzymatic decomposition of H_2O_2 and C were more rapid in ice at -11° or -18° C than at +1°C; both followed first-order kinetics. That of H_2O_2 was partly dependent on FeCl or CuCl, but that of C was independent of metal ions. For C the pH optimum was 7.2, and the pH effect was not parallel to metal ion effects on the kinetics. The authors concluded that in ice there was a different and previously unsuspected mechanism for degradation of C.

Some further data are given in Table 36.

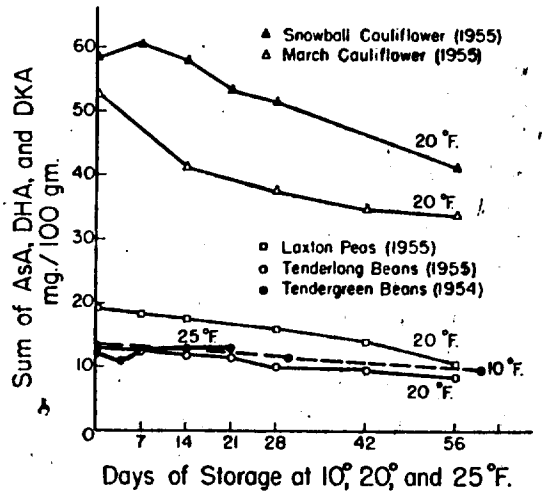

Figure 8. Effect of Storage on the Sums of Reduced Ascorbic Acid, Dehydroascorbic Acid, and Diketogulonic Acid in Cauliflower, Peas, and Bush Beans at 20°F; and at 10° and 25° F for Bush Beans (2063)

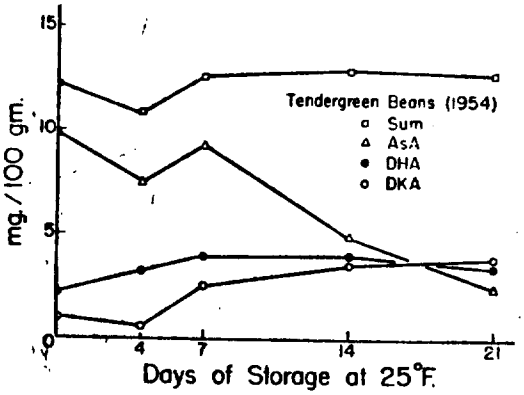

Figure 9. Effect of Storage at 25° F on the AsA, DHA, DKA Content of (1954) Tendergreen Bush Beans Graphed Separately and as a Sum of the Three Compounds (2063)

In 1957 Dietrich et al. (2063) related the storage history of frozen vegetables to contents of reduced C, but noted that this measure was useful only if the original contents were known. More useful was the summation of reduced C and its oxidation products dehydro-C and diketogulonate, especially for green beans (Figures 6 -10).

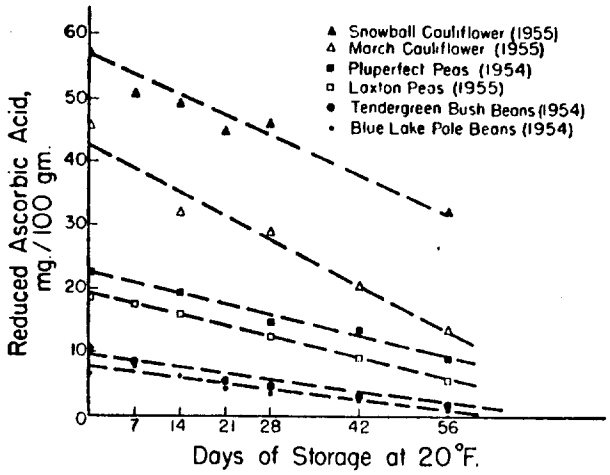

Figure 6. Loss of Reduced Ascorbic Acid (AsA) (indophenol method) in Cauliflower, Peas and Green Beans at 20°F (2063)

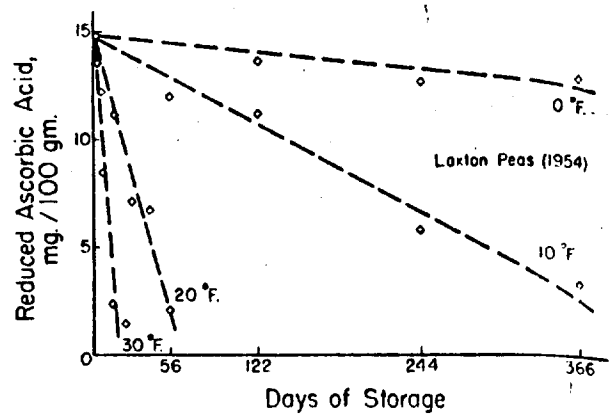

Figure 7. Loss of Reduced Ascorbic Acid (AsA) (indophenol method) in Laxton Peas (1954) at 0°, 10°, 20°, and 30°F (2063)

Table 35. Effect of Storage on Vitamin C Content of Foods

Food[a]	Temperature	Time	% Loss	Reference
Lemons & oranges	45-50°F	2 months	0	0753
		10 months	10-30	
		3 months	15	
Apples	45°F	6 months	25	0753
		12 months	50	
Cabbage	Room temp.	1 month	25	0753
	45-50°F		10	
Spinach	Room temp.	Few days	50	0753
	37-38°F		0	
Kale, spinach, collards, turnip greens, rape	70°C[b]	7 days	90	2690
	32°F		3-30	
Potatoes	Not given	3 months	50	2690
Orange juice, fresh		3 days	<1	5025
Orange juice, frozen	24-28°C	8 days	<1	
Orange juice, canned		8 days	<1	
Ascorbic acid soln[c]		8 days	<1	
Orange juice	4-5°C	2 weeks	9	2690
Apple juice, fortified	70°F	12 months	19-27	2690
Prune juice, fortified	Room temp.	3 months	30-50	2690
Fruit drinks, opened	Refrigerator	7 days	10-20	2690
Breakfast cereal, fort.	Room temp.	9 months	40	2690
Cocoa, powdered, fort.	75°F	12 months	10-15	2690

[a]Ascorbic acid oxidase occurs in a variety of food plants and may be responsible for significant losses if not inactivated during processing (2690).

[b]May be misprint in original article; may be 70°F.

[c]0.05% aqueous solution.

Table 34. Vitamin C Content of Fresh Fruits and Vegetables (0753) (Cont'd)

Horseradish	Unknown	Garden fresh	1.224
Kale	Scotch	Market (Autumn)	1.877
Kale	Scotch	Market (Winter)	1.011
Kumquat	Unknown	Market	0.549
Leek (blanched)	Unknown	Market	0.180
Lettuce	California	Market	0.133
Lettuce	Hothouse	O. S. U.	0.152
Lettuce	Mignonette	Garden fresh	0.205
Lettuce	Tennis Ball	Garden fresh	0.553
Lemon	"Sunkist"	Market	0.64
Lima Bean	Hopi	Garden fresh	0.296
Lime	Unknown	Market	0.398
Melon	Honeydew	Market	0.942
Melon, Musk	Polish No. 1	Garden fresh	0.374
Mustard Greens	Unknown	Market	1.790
Nasturtium Seeds	Unknown	Freshly picked	1.089
New Zealand Spinach	Unknown	Garden fresh	0.255
Okra	Long Green	Garden fresh	0.147
Onion	Brown	Mature	0.144
Orange	"Florida"	Market	0.360
Orange	Navel	Market	0.480
Parsley	Paramount	Garden fresh	1.094
Parsnip	Hollow Crown	Garden fresh	0.326
Paw-Paw	Wild	Freshly picked	0.360
Peach	Elberta	Market	0.084
Pear	Kieffer	Freshly picked	0.067
Pea	California Telephone	Market	0.214
Pea	Little Marvel	Garden fresh	1.028
Pepper (hot)	Hungarian Wax	Market	1.272
Pepper (green)	Windsor A	Garden fresh	0.976
Pepper (ripe)	Sunnybrook	Garden fresh	2.383
Potato	Early Ohio	Market	0.282
Radish	White Icicle	Market	0.261
Rhubarb	Flare	Garden fresh	0.444
Sage	Unknown	Garden fresh	0.250
Salsify	Sandwich Island	Market	0.073
Soy Bean (green)	Jogun	Freshly shelled	0.428
Soy Bean (green)	Bansei	Freshly shelled	0.404
Spinach	Unknown	Market	0.492
Spinach	Unknown	Garden fresh	1.123
Squash	White Bush	Market	0.232
Strawberry	Clermont	Freshly picked	1.429
Sweet Corn	Golden Bantam	Market	0.428
Sweet Corn	Stowell's Evergreen	Market	0.108
Sweet Potato	Jersey	Market	0.326
Swiss Chard (leaves)	Fordhook	Garden fresh	0.321
Swiss Chard (stems)	Fordhook	Garden fresh	0.075
Tangerine	Unknown	Market	0.484
Tomato (green)	Stokesdale	Garden fresh	0.180
Tomato (ripe)	Stokesdale	Garden fresh	0.36–0.57
Tomato (ripe)	Master Marglobe	Garden fresh	0.558
Tomato (ripe)	Small German Sugar	Garden fresh	0.72
Tomato (ripe)	Yellow Plum	Market	0.262
Tomato (ripe)	Unknown	Hothouse	0.200
Turnip	Shogoin	Garden fresh	0.240
Turnip Greens	Shogoin	Garden fresh	1.214
Zucchini	Unknown	Market	0.237

Ascorbic acid is very unstable in many foods and its behavior in this respect has been studied more than that of other vitamins. The literature is voluminous (2690).

Significant vitamin C changes in the living food plant have been noted. West Indian cherries, for example, when partly ripe have a vitamin C content of 1135–1916 mg/100 grams; when fully ripe, however, the content is only 577–1246 mg/100 grams (0329). Sweet corn and green peas have their maximum amount of vitamin C during the tender stage and the level decreases as the seed matures. In general, most leafy and root vegetables have their greatest concentration of vitamin C during the period of most rapid growth (0753).

Vitamin C losses after harvest and in the marketplace for a large variety of fresh fruits and vegetables are presented in Table 34.

Table 34. Vitamin C Content of Fresh Fruits and Vegetables (0753)

Material	*Variety*	*Source*	*Milligrams of ascorbic acid per gram fresh weight*
Apple	Jonathan	Market	0.044
Apple	Wolfe River	Freshly picked	0.099
Asparagus	Mary Washington	Garden fresh	1.007
Asparagus	Unknown	Market	0.45
Avocado	Unknown	Market	0.270
Banana	Unknown	Market	0.284
Beans, Snap	Tendergreen	Garden fresh	0.453
Beans, Snap	Unknown	Market	0.195
Beans, Snap	Yellow Wax	Market	0.236
Broccoli	Italian Sprouting	Garden fresh	1.375
Broccoli	Italian Sprouting	Market	0.801
Brussel's Sprouts	Unknown	Market (Autumn)	1.846
Brussel's Sprouts	Unknown	Market (Winter)	1.19
Cabbage	Average for thirty varieties	Garden fresh	1.002
Carrot	Tendersweet	Garden fresh	0.111
Cauliflower	Primosnow	Garden fresh	0.960
Celery (blanched)	Salt Lake	Garden fresh	0.104
Chinese Cabbage	Unknown	Market	0.269
Chives	Unknown	Garden fresh	0.543
Cherry	Early Richmond	Freshly picked	1.386
Cress	Water-cress	Freshly picked	1.875
Cress	Water-cress	Market	0.600
Cucumber	Chinese	Garden fresh	0.107
Currant	Red Lake	Freshly picked	0.245
Dandelion	"Greens" (Spring)	Freshly picked	1.546
Dandelion	"Greens" (Summer)	Freshly picked	0.303
Egg Plant	Black King	Garden fresh	0.121
Endive (blanched)	Bavarian	Garden fresh	0.054
Endive (green)	Bavarian	Garden fresh	0.130
Garlic	Unknown	Market	0.139
Gooseberry	Poorman	Freshly picked	0.165
Grapefruit	Unknown	Market	0.364
Grape	Thompson Seedless	Market	0.055

I. Breakdown

When Peters and Davenport reviewed progress in 1938 (6417) the important pathways of degradation of C were still in debate. Glutathione had been confirmed as a stabilizer for C in blood. Kon had observed photodegradation of C in milk. Others, in Germany, had found that C deaminated amino acids.

In 1941 King and Tressler (4337) made the following observations:

1. All plant and animal foodstuffs lost C by respiratory enzyme actions when stored as natural products. Vegetables and fruits lost about one-half their content of C during a season of weeks or months while remaining marketable, even if kept in cold store. Meat lost its C "steadily" but, if frozen, might retain enough "to be fully protective." Milk lost about 10%/day, but "market milk" lost C much more rapidly owing to contact with copper.
2. Dehydration destroyed virtually all C by enzymic oxidation. Sulfites, drying *in vacuo*, powdering of milk, assisted retention of C.
3. Blanching helped retain C by destroying oxidative enzymes.
4. Crushing, slicing, freezing and thawing, destroyed C except in highly acid foodstuffs.
5. Pasteurization destroyed only "slight" amounts of C.
6. Cooking destroyed varying amounts of C. Losses during careful steaming and boiling were 10-25% ; during baking, frying and roasting 30-60%; and during any careless form of cooking could be 50-90%.
7. Canning losses could be less than 20% and often were less than 10%. Even homogenized babyfoods could retain 35-65% of the original C.
8. Fermenting and pickling were inherently destructive of C but could be regulated to minimize destruction, e.g., sauerkraut lost about 50% of its C in fermentation, and if canned, it lost 20% of the remainder; otherwise, loss during storage was slow and steady.